



Grant Number: 1R01AI098775-01

Principal Investigator(s):

Maria Elena Bottazzi
PETER J HOTEZ (contact), PHD
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Helen Shepherd
Business Manager
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Budget Period: 05/04/2012 – 04/30/2013

Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$1,501,840 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Shellie M. Wilburn

Additional information follows

SECTION I – AWARD DATA – 1R01AI098775-01**Award Calculation (U.S. Dollars)**

Salaries and Wages	\$190,031
Fringe Benefits	\$52,093
Equipment	\$298,503
Supplies	\$65,231
Travel Costs	\$20,000
Other Costs	\$15,000
Consortium/Contractual Cost	\$636,563

Federal Direct Costs	\$1,277,421
Federal F&A Costs	\$224,419
Approved Budget	\$1,501,840
Federal Share	\$1,501,840
TOTAL FEDERAL AWARD AMOUNT	\$1,501,840

AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$1,501,840
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SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
1	\$1,501,840	\$1,501,840
2	\$1,154,596	\$1,154,596
3	\$1,134,359	\$1,134,359
4	\$1,165,726	\$1,165,726
5	\$1,165,855	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1741613878A1
Document Number:	RAI098775A
Fiscal Year:	2012

IC	CAN	2012	2013	2014	2015	2016
AI	8472315	\$1,501,840	\$1,154,596	\$1,134,359	\$1,165,726	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC: M51C B / OC: 414A / Processed: P11 04/27/2012

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI098775-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 1R01AI098775-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- The grant program legislation and program regulation cited in this Notice of Award.
- Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at '<http://grants.nih.gov/grants/policy/awardconditions.htm>' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI098775-01

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

Restriction: Under governing PHS Policy, Federal funds administered by the Public Health Service (PHS) shall not be expended for research involving live vertebrate animals without prior approval by the Office of Laboratory Animal Welfare (OLAW) of an Assurance to comply with the PHS Policy on Humane Care and Use of Laboratory Animals and the project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The present award is being made without currently valid verification of IACUC approval for the portion of this project being completed in CHINA with the following restriction: No activities that involve live vertebrate animals may be conducted at Frontier Biosciences located in CHINA pending acceptance by the NIH awarding component of verification of IACUC approval. The Program Officer has approved the funding of this application without the portion of Frontier Biosciences located in CHINA in year 05 as the project is viable without it. No funds may be expended for the foreign site pending the resolution of internal administrative issues. Once these issues have been resolved, this award may be revised to include the study originally planned for the foreign site. Failure to comply with this special condition can result in suspension and/or termination of this award, withholding of support, audit disallowances, and/or other appropriate action.

The budget period anniversary start date for future year(s) will be May1.

None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice: <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-035.html> Therefore, this award and/or future years are adjusted accordingly, if applicable.

This award includes funds awarded for consortium activity with NY Blood Center in the amount of \$445,904 (\$263,070 direct costs + \$182,834 facilities and administrative costs);

This award includes funds awarded for consortium activity with the University of Texas Medical Branch in the amount of \$190,659 (\$124,614 direct costs + \$66,045 facilities and administrative costs).

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2011/nihgps_ch15.htm.

The research proposed in this grant may involve Select Agents and/or Highly Pathogenic Agents. Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving approval from the NIAID, NIH Program Official. Awardee is responsible for having its subcomponent/subcontractor comply with the requirements pertaining to the use of Select Agents and/or Highly Pathogenic Agents. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that, under some circumstances, may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>), and your Institutional Biosafety Committee (IBC) or equivalent body, or appropriate designated institutional biosafety official. If there is ambiguity in the BMBL guidelines and/or there is disagreement among the BMBL, an institutional committee or institutional official, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

For work with Select Agents performed in the U.S. provide documentation of Registration status of all domestic organizations/entities where Select Agent(s) will be used. For work with Select Agents performed in a non-U.S. country prior NIAID approval is required.

Please be advised that changes in the use of a Select Agent will likely be considered a change in scope and, therefore, require NIH awarding office prior approval.

Please contact Donna Sullivan at 301-594-6361 or at dsullivan@niaid.nih.gov if there is a need to be provided additional assistance in a grant-related issue.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Mary Ann Anderson-garlic
Email: mgarlic@niaid.nih.gov **Phone:** 301-451-3680 **Fax:** 301-493-0597

Program Official: Rachelle Salomon
Email: salomonra@mail.nih.gov **Phone:** 301-402-2202 **Fax:** 301-496-8030

SPREADSHEET SUMMARY

GRANT NUMBER: 1R01AI098775-01

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	\$190,031	\$122,034	\$174,866	\$57,811	\$54,221
Fringe Benefits	\$52,093	\$33,453	\$47,936	\$15,848	\$14,864
Equipment	\$298,503				
Supplies	\$65,231	\$60,485	\$71,400	\$10,000	\$10,126
Travel Costs	\$20,000	\$10,000	\$20,000	\$5,000	\$5,000
Other Costs	\$15,000	\$15,000	\$95,000	\$579,807	\$609,000
Consortium/Contractual Cost	\$636,563	\$775,547	\$490,684	\$114,229	\$75,434
TOTAL FEDERAL DC	\$1,277,421	\$1,016,519	\$899,886	\$782,695	\$768,645
TOTAL FEDERAL F&A	\$224,419	\$138,077	\$234,473	\$383,031	\$397,210
TOTAL COST	\$1,501,840	\$1,154,596	\$1,134,359	\$1,165,726	\$1,165,855

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	57.3%	57.3%	57.3%	57.3%	57.3%
F&A Cost Base 1	\$342,355	\$240,972	\$409,202	\$668,466	\$693,211
F&A Costs 1	\$196,169	\$138,077	\$234,473	\$383,031	\$397,210
F&A Cost Rate 2	56.5%				
F&A Cost Base 2	\$50,000				
F&A Costs 2	\$28,250				

PI: HOTEZ, PETER J	Title: RBD recombinant protein-based SARS vaccine for biodefense	
Received: 05/26/2011	FOA: AI11-014	Council: 01/2012
Competition ID: ADOBE-FORMS-B1	FOA Title: PARTNERSHIPS FOR BIODEFENSE (R01)	
1 R01 AI098775-01	Dual:	Accession Number: 3392091
IPF: 481201	Organization: BAYLOR COLLEGE OF MEDICINE	
Former Number:	Department: Pediatrics	
IRG/SRG: ZAI1 RGK-M (J2)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 1,048,503 Year 2: 749,999 Year 3: 750,000 Year 4: 750,000 Year 5: 749,999	Animals: Y Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	New Investigator: N Early Stage Investigator: N
<i>Senior/Key Personnel:</i>	<i>Organization:</i>	<i>Role Category:</i>
Peter Hotez M.D.	Baylor College of Medicine	PD/PI
Shibo Jiang M.D.	New York Blood Center	MPI
Maria Bottazzi Ph.D	Baylor College of Medicine	MPI
Sara Lustigman Ph.D	New York Blood Center	Co-Investigator
Chien-Te Tseng Ph.D	The University of Texas Medical Branch	Co-Investigator
Lanying Du Ph.D	New York Blood Center	Co-Investigator
Bin Zhan M.D.	Baylor College of Medicine	Co-Investigator
Tehsheng Chan PhD	University of Texas Medical Branch	Co-Investigator

Appendices

App1 a research paper related to rbd of sars-cov s protein, App2 review article vaccine development, App3 patents directly relevant to the project, Appendix summary sheet

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. * TYPE OF SUBMISSION

☐ Pre-application ☐ Application ☒ Changed/Corrected Application

2. DATE SUBMITTED

Applicant Identifier

35116-I

4. a. Federal Identifier

GRANT10878569

b. Agency Routing Identifier

5. APPLICANT INFORMATION

* Organizational DUNS: 051113330

* Legal Name: Baylor College of Medicine

Department: Office of Research

Division: NA

* Street1: One Baylor Plaza

Street2:

* City: Houston

County / Parish:

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 77030-3411

Person to be contacted on matters involving this application

Prefix: * First Name: Miki

Middle Name:

* Last Name: Gillis

Suffix:

* Phone Number: 832-824-2283

Fax Number: 713-790-1345

Email: myates@bcm.edu

6. * EMPLOYER IDENTIFICATION (EIN) or (TIN): 1741613878A1

7. * TYPE OF APPLICANT:

O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type

☐ Women Owned☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award☐ B. Decrease Award☐ C. Increase Duration☐ D. Decrease Duration☐ E. Other (specify):* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY:

National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

RBD recombinant protein-based SARS vaccine for biodefense

12. PROPOSED PROJECT:

* Start Date

* Ending Date

05/01/2012

04/30/2017

* 13. CONGRESSIONAL DISTRICT OF APPLICANT

TX-007

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: * First Name: Peter

Middle Name: J

* Last Name: Hotez

Suffix: M.D.

Position/Title: Professor

* Organization Name: Baylor College of Medicine

Department: Pediatrics

Division: College of Medicine

* Street1: One Baylor Plaza, BCM320A

Street2:

* City: Houston

County / Parish:

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 77030-3411

* Phone Number: 832-824-2283

Fax Number:

* Email: hotez@bcm.edu

15. ESTIMATED PROJECT FUNDING a. Total Federal Funds Requested 6,265,449.00 b. Total Non-Federal Funds 0.00 c. Total Federal & Non-Federal Funds 6,265,449.00 d. Estimated Program Income 0.00	16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS? a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON: DATE: b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR ☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW
17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001) ☒ * I agree * The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.	
18. SFLLL or other Explanatory Documentation Add Attachment Delete Attachment View Attachment	
19. Authorized Representative Prefix: * First Name: Helen Middle Name: * Last Name: Shepherd Suffix: * Position/Title: Executive Director, Office of Research * Organization: Baylor College of Medicine Department: Office of Research Division: NA * Street1: One Baylor Plaza, BCM310 Street2: * City: Houston County / Parish: * State: TX: Texas Province: * Country: USA: UNITED STATES * ZIP / Postal Code: 77030-3411 * Phone Number: 713-798-1297 Fax Number: 713-798-6990 * Email: spo@bcm.edu * Signature of Authorized Representative Helen Shepherd * Date Signed 05/26/2011	
20. Pre-application Add Attachment Delete Attachment View Attachment	

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Appendix

Number of Attachments in Appendix: 4

Project/Performance Site Location(s)**Project/Performance Site Primary Location**☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: BAYLOR COLLEGE OF MEDICINE

DUNS Number: 0511133300000

* Street1: ONE BAYLOR PLAZA

Street2:

* City: HOUSTON

County:

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 77030-3411

* Project/ Performance Site Congressional District: TX-007

Project/Performance Site Location 1☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: New York Blood Center

DUNS Number: 0732718270000

* Street1: 310 East 67 Street

Street2:

* City: New York

County:

* State: NY: New York

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 10065-6275

* Project/ Performance Site Congressional District: NY-014

Project/Performance Site Location a☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The University of Texas Medical Branch

DUNS Number: 8007711490000

* Street1: 301 University Boulevard

Street2:

* City: Galveston

County: Galveston

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 77555-0156

* Project/ Performance Site Congressional District: TX-014

Project/Performance Site Location(s)

Project/Performance Site Location 3

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Texas Childrens Hospital

DUNS Number: 0746153940000

* Street1: 1102 Bates Street

Street2:

* City: Houston

County:

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 77030-2399

* Project/ Performance Site Congressional District: TX-007

Additional Location(s)

Add Attachment

Delete Attachment

View Attachment

RESEARCH & RELATED Other Project Information1. * Are Human Subjects Involved? ☒ Yes ☐ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☒ NoIf yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6If no, is the IRB review Pending? ☒ Yes ☐ NoIRB Approval Date: Human Subject Assurance Number: 2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ NoIACUC Approval Date: Animal Welfare Assurance Number 3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No4.b. If yes, please explain: 4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No4.d. If yes, please explain: 5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No5.a. If yes, please explain: 6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No6.a. If yes, identify countries: 6.b. Optional Explanation: 7. * Project Summary/Abstract 8. * Project Narrative 9. Bibliography & References Cited 10. Facilities & Other Resources 11. Equipment 12. Other Attachments ☒

Abstract

The 2002-2003 pandemic of severe acute respiratory syndrome (SARS) posed an enormous threat to global public health and the social and economic stability. Its causative pathogen, the SARS-associated coronavirus (SARS-CoV), has been classified by NIAID as a Category C Priority Pathogen. SARS outbreaks remain a serious concern mainly due to possible zoonotic reintroduction of SARS-CoV into humans, accidental release from a laboratory or deliberate spreading of the virus by a bioterrorist attack. Therefore, an effective and safe vaccine is urgently needed for preventing future SARS outbreaks and for biodefense preparedness. We have identified a highly promising lead candidate vaccine antigen, the receptor binding domain (RBD) of the SARS-CoV spike (S) protein that contains the major neutralizing epitopes and can induce potent neutralizing antibody response and protection in animals against SARS-CoV infection. To rapidly translate our initial proof of concept findings into a solid platform of clinical trials, a consortium of experts was put together consisting of scientists from Baylor College Medicine, the new home of Sabin Vaccine Institute's product development partnership (BCM-Sabin), the New York Blood Center (NYBC) and the University of Texas Medical Branch (UTMB), and in partnership with industrial partners and non-profit organizations. The specific aims of this proposal are: **(1) Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate.** The rRBD protein will be expressed in bacteria and yeast expression systems and one of these expression systems will be selected for subsequent studies based on yields, purity, stability, antigenicity, functionality, immunogenicity, and efficacy of the rRBD protein. The immunization regimens will be optimized and the ability of rRBD protein to induce cross-neutralizing antibody response, cross-protection and long-term immune responses and protection will be assessed. **(2) Process development, characterization, formulation and stability profiling.** A scalable and reproducible fermentation process for rRBD (10 liter scale) and a purification process using chromatographic technologies will be developed. Reproducibility will be confirmed. The specific product quality assays and vaccine formulations with alum and/or glucopyrranosyl lipid A (GLA), an innate adjuvant, will be developed. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. **(3) Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation.** The cell bank production, production processes and the formulation technology for the rRBD-based SARS vaccine will be transferred to Walter Reed Army Institute of Research (WRAIR) pilot facility for 60-L scale GMP manufacture, formulation and fill and finish. The clinical lots will be released by Sabin-Texas and following a pre-IND meeting with the U.S. FDA, GLP toxicology will be initiated at Frontier Biosciences, a Maryland-based contractor.

Project Narrative

SARS-CoV is the first new human infectious disease agent of this century classified by NIAID as a Category C Priority Pathogen, and SARS outbreaks still remain a serious global concern mainly due to possible zoonotic reintroduction of SARS-CoV into humans or accidental release from a laboratory or deliberate spreading of the virus by a bioterrorist. We have identified a highly promising lead candidate vaccine antigen, the receptor binding domain (RBD) of the SARS-CoV spike (S) protein that can induce potent neutralizing antibody response and protection against SARS-CoV infection. Our objective is to develop a highly effective and safe recombinant RBD-based SARS vaccine that can be used in humans for prevention of future SARS outbreak and for biodefense preparedness.

FACILITIES & RESOURCES

Baylor College of Medicine

Laboratory:

The Hotez lab occupies approximately 8500 square feet of well-equipped bench laboratory space in the recently renovated Feigin Research building at Texas Children's Hospital. A major expansion of which (an addition 8 floors has been added) was completed in 2009. The laboratory is equipped with -80° and -20° freezers as well as adequate bench space. Expression capabilities include *Pichia pastoris*, *Saccharomyces cerevisiae*, *Escherichia coli*, Baculovirus and mammalian cell culture expression systems. Generation and characterization of research cell banks. For Process Development and studies designed to optimize large-scale protein production include fermentation capabilities from 1-10L scales. Facility is capable of developing and optimizing protein purification processes. The optimal purification scheme is dependent upon the properties of the particular protein, which is being purified. Procedures may be included, but are not limited to ion exchange chromatography, (anion or cation), hydrophobic interaction chromatography, hydroxyapatite, reverse phase and size exclusion, IMAC and affinity chromatography. The laboratories are fully suited to perform biochemical characterization (testing for identity, purity, conformation, integrity, stability, consistency, antigenicity and residual host cell proteins) of in-process and final purified bulk protein samples. Formulation studies are also performed (testing for compatibility, stability, immunogenicity and potency) with multiple adjuvants. All process development and analytical work performed under a strictly-regulated environment with highly-trained individuals performing operations according to formal standard operating procedures, batch production records and protocols. In addition, the lab has access to all the Texas Children's core facilities and expertise as listed below.

Animal:

Baylor College of Medicine (BCM) maintains an animal program fully accredited by the American Association for the Accreditation of Laboratory Animal Care since October 26, 1995. The Center for Comparative Medicine (CCM) administers the program of veterinary care. All animals are maintained in accordance with the provisions of the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. Animals are housed in six central animal facilities. These animal facilities are located throughout the medical center and together comprise 56,000 square feet of conventional animal housing space. In addition CCM maintains a 7,400 square feet mouse barrier facility. Animals in all animal facilities are observed daily by members of CCM staff. The Center has a staff of 5 full time veterinarians. Three of the veterinarians are diplomats of the American College of Laboratory Animal Medicine (ACLAM) and have extensive experience with laboratory animal species. A comprehensive diagnostic laboratory is maintained by the veterinary staff and headed by a board certified veterinary pathologist.

In vivo imaging:

Animal imaging modalities including bioluminescence (IVIS Xenogen system), ultrasound, in vivo fluorescence, PET, CT/SPECT and MRI scanning are all immediately available in either the Feigin Center animal facility or in the imaging center at Baylor College of Medicine. All animals are manipulated under animal protocols reviewed and approved by the Baylor Animal Research Committee.

Computer:

Laboratories and offices have exclusive use Pentium-based personal computers, complete with word processing software, extensive scientific software, database programs, spreadsheet programs, electronic mail, scheduling, and Internet browsers. Computers are connected to a campus-wide network to provide access to central services such as file servers, data base servers, UNIX servers, printers, and film recorders, as well as to facilitate the sharing of information between colleagues both locally and long distance via the Internet. A DEC 8060 computer is available for statistical analysis plotting. Full time IT and data management support is provided by Baylor College of Medicine.

Office:

The principal investigators has adequate office space adjacent to his laboratory.

Other:

Texas Children's Hospital (TCH) is located in the Texas Medical Center (TMC) that comprises 675 acres and is

the world's largest medical center. The TMC encompasses 42 not-for profit institutions dedicated to the highest quality patient care, research, and education. These institutions include 13 hospitals, two medical schools, four schools of nursing, a school of public health, a college of pharmacy, two graduate schools of biomedical sciences, and a medical library. More than 5.4 million patient visits were recorded in 2000, and more than \$573 million in funded research is conducted annually. Additionally, two other major universities with vigorous programs in biological sciences are within easy access; Rice University is adjacent to the TMC, and the University of Houston (UH) is 3 miles away. The UH College of Pharmacy is located in the TMC. Other resources available to the investigator include a Medical Illustration Department, which can meet all audio visual or printed material needs, and the Houston Academy of Medicine/TMC Library. TCH also has a 75 member Biomedical Engineering Department, and an excellent machine shop and electronic shop, which are available as-needed basis.

New York Blood Center

Laboratory: LABORATORY OF VIRAL IMMUNOLOGY

General laboratory area (2,946 feet²) including tissue culture section; tissue culture laboratories (2 BSL-2 facilities, 760 feet²); walk-in refrigerator (98 feet²); area for centrifuges (120 feet²); BSL-3 facility (400 feet²) with air locks, limited access corridors and a ventilation system providing directional air flow incorporating high efficiency HEPA filters with water resistant interior surfaces which are sealed.

Clinical:

N/A

Animal:

N/A

Computer:

Computers: Two Silicon Graphics Octane, one HP xw 9300 Workstation, eight desktop computers, and two IBM ThinkPad T30. Printers: HP Color LaserJet 4700dn, HP LaserJet 4200n, HP Officejet Pro 8500.

Office:

3 offices (420 feet²).

Laboratory: LABORATORY OF MOLECULAR PARASITOLOGY

Molecular Parasitology at the Lindsley F. Kimball Research Institute of the New York Blood Center consists of three labs and occupies approximately 955 square feet, containing approximately 80 linear feet of active bench space (room 1, 655 square feet and room 2, 300 square feet). The largest laboratory is fully equipped for molecular biology research, whereas the second lab is mostly committed to research involved with tissue culture and RNA work.

Clinical:

N/A

Animal:

N/A

Computer:

There are presently 4 PC computers (Pentium) and 1 Macintosh computers (Power Macintosh G4) available in this department. They contain programs for DNA and protein analysis (Vector NTI Suite, DNASIS/PPROSIS, DNA Star), word-processing, data management, desktop publishing, bibliographic database, digital imaging software, web browsers and graphics presentations. All the computers have access to the Internet and Color Printer.

Office:

There are 2 offices (total of 225 square feet) for researchers. Photocopy, scanning and fax equipment is also available in this space.

Other:**The following Core facilities are available to us:****1. Flow Cytometry Laboratory (FCL)**

Staff: Wu He, Ph.D.

The Flow Cytometry Laboratory (FCL) provides investigators with equipment and support for cell sorting (separation) from single cell suspension using fluorescence and for acquisition and analysis of flow cytometric data. For analysis purposes, the FCL is equipped with a bench top alignment-free multipurpose analyzer from BD Immunocytometry Systems (FACSCanto). An additional FACSCalibur analyzer is also available. The laser configurations on these instruments allow for analysis of up to 6 colors on the FACS Canto and up to 4 colors on the FACSCalibur with 488 nm and 633 nm-excited dyes. A number of applications, including the multicolor analysis of cell phenotype, gene expression and cell cycle may be performed. For cell sorting, the FCL is equipped with a MoFlo high-speed cell sorter (DAKO Cytomation). This instrument provides high-speed, high purity (up to 99.0%) multilaser cell sorting and is capable of sterile sorts of single cells. The sorter is operated by FCL staff only.

2. Radioisotope suite:

Staff: Rawlinson Isaac

The radioisotope suite is a shared facility of the Lindsley F. Kimball Research Institute. The suite occupies 400 square feet and is equipped with seven scintillation counters, including the LKB Betaplate 1205 and a Tomtec 96 cell Harvester.

3. Laboratory of Electron Microscopy:

Staff: Yelena Oksov, M.S, Electron Microscopist

The facility provides transmission electron microscopy (TEM) services, including full specimen preparation through fixation to embedding, thin and ultrathin sectioning and staining and analyzing results of experiments. The electron microscopic methods include:

1. Conventional electron microscopy of the cells and tissues.
2. Negative staining to study particles and molecules in solution or in suspension.
3. Immunoelectron microscopy (IEM) to visualize the location of antigenic agents.

Equipment: Electron Microscope, EM 410, Philips Electronic Instruments/FEI equipped with a Digital Camera; Advantage HR Digital CCD Camera System; Ultramicrotome, MT-X, RMC; High Vac Evaporator, Denton Vacuum; Camera System, MP-4 Standard, Polaroid.

4. Laboratory of Confocal Microscopy:

Staff: Lyudmil Angelov, MS.

With the Zeiss LSM 510 META Confocal Microscope, a laser scanning fluorescent microscope, the laboratory provides three- and four-dimensional images of living cells and tissue. Researchers can study cellular structures and determine the location of multiple proteins simultaneously within the cell. Observed over time, the images make a film of the movement of proteins within the cell.

5. Shared Instrumentation Laboratory

Staff: Richard Sanford

Tri-Carb 2900RT (Packard BioScience); Minaxi Auto-Gamma 5530 (Packard BioScience); Beta plate Flatbed Scanner 1205-001 (PerkinElmer); Storm 860 (Molecular Dynamics/Amersham Bioscience); Konica SRX-101A Film Developer; Alpha Beta Sample Counter 3030 (Ludlum); Savant SpeedVac System; U-2000 UV/Vis Spectrophotometer (Hitachi); Vacumatic Stage 3 Autoclave 3023 (Steris); Better Built Glasswasher (LBR Scientific Inc); Irradiator (Isomedix); Phosphoimager (Molecular Dynamics); ABI PRISM 7300 sequence detection system (PE Applied Biosystems); Biacore 3000 (Biacore, Piscataway, NJ).

6. Laboratory for Animal Research Services (LARS)

Staff: Kathy Tang

The Animal Services facility of the New York Blood Center is a specifically designated unit to house small laboratory animals. These animal quarters are under the direction of a full-time supervisor who works closely with a consultant Veterinarian. The facility is registered with the U.S. Department of Agriculture, Animal Health Division #21-8, and is fully accredited by the American Association of Laboratory Animal Care.

Scientific environment: The project aims to be executed at the New York Blood Center are supported by the collegial environment at the Lindsley F. Kimball Research Institute (LFKRI) of the New York Blood Center and the close contact and collaboration between the groups of Dr. Shibo Jiang (PI#2 at NYBC), head of the Viral Immunology Laboratory and Dr. Sara Lustigman (Co-Investigator), head of Molecular Parasitology laboratory and. Both laboratories are located in the same building. Both PIs have common interest in developing vaccines against different pathogens using common and innovative adjuvants, one of which was discovered and is being developed for clinical use by both groups, thus having carried out several collaborative projects since 2005. Their past collaborative research resulted in 6 published manuscripts, and others are in preparations for publications. They have regular meetings at least once a week to discuss their collaborative projects. Their lab members have often shared the equipments, exchanged reagents and provided technical assistance for each other. They will report their progress of the project through the laboratory seminar on Wednesday as well as by having teleconference meetings and in person with the group at Sabin-Texas. Infrastructural support for research is provided at LFKRI in the form of several core facilities as indicated in the section of the Equipment located in the Core Science Laboratories.

University of Texas Medical Branch

Laboratory: Dr. Tseng has approximately 1,200 sq.ft. of laboratory space. Readily convenient to his laboratory are the vivarium, tissue culture room, and glassware and sterilizing services.

Office: Dr. Tseng has a up-to-date computer in his office and the laboratories are equipped with PC's for data retrieval and record-keeping

Other: On-campus support groups include a computation center in which hardware and software are available for data reduction and statistical analysis, a common darkroom facility, column chromatography, spectrophotometric analysis and routine biochemical and cellular assays. In addition, housed on the sixth floor of the Medical Research Building is the Sealy Center for Molecular Science. The Sealy Center for Molecular Science offers plasmid DNA preparation, plasmid DNA transformation and fragments and oligonucleotide purification vector/plasmid DNA construction, DNA sequence analysis, genomic and cDNA library construction and screening, bacterial expression of recombinant genes, polymerase chain reaction technology and expertise in various types of mutagenesis. Dr. Tseng will conduct the proposed studies with SARS-associated coronavirus (SARS-CoV) in the campus' BSL-3 laboratory.

BIOHAZARDS AND BIOSAFETY:

The BSL-3 laboratory:

Most of the UTMB BSL-3 laboratories are located in the Keiller building as part of the Center for Biodefense and Center for Tropical Diseases. Laboratory space in Keiller Building includes ten standard BSL-2

laboratories (total space approximately 8,000 ft²), plus three special BSL-3 laboratories (each 282 ft²) for work with hazardous viral agents. The BSL-3 laboratories have been inspected by both CDC and USDA/APHIS and are approved for work with "select agents." Core facilities include: a new, 6,550 ft² animal biosafety level 2 and 3 facility; a 750 ft² level 2 arthropod containment facility; a fully equipped Electron Microscopy Laboratory; and a darkroom equipped to develop X-Ray film. Additionally, UTMB has recently provided a new BSL-3 lab (235 ft²) for SARS-related studies. This new lab, located at the fifth floor of Mary Moody Northern Pavilion, is equipped with two tissue culture hoods, two CO₂ incubators, one refrigerated tabletop centrifuge, and a computer. The P.I. of this project will have access to this new lab.

All experiments that use infectious SARS-CoV in the preliminary study have been done in one BSL-3 lab in the Keiller building. This BSL-3 lab has one tissue culture hood and one CO₂ incubator. Dr. Tseng is highly trained and experienced in working with BSL-3 infectious agents.

Those who work on SARS-CoV will be under medical surveillance so that if any febrile or lower respiratory illness develops, they will be evaluated medically. The medical evaluation will be by the infectious disease fellow on call in a negative pressure room that contains specific protocols for suspected SARS patients.

Inactivation of SARS-CoV: We have access to a gammacell⁶⁰ Cosource (Model# 109A, JL Shepherd & Associates, San Fernando, CA) to provide an alternate mode of inactivation to the conventionally employed detergent or chaotropic ion extractions. Samples that require further experimental steps outside of a BSL-3 lab will be irradiated by ⁶⁰Co to complete inactive SARS-CoV infectivity. The kill curve of SARS-CoV by gamma irradiation has been determined, in which a single dose of 10⁶ rads is sufficient to completely inactivate 1 x 10⁷ TCID₅₀/ml.

UTMB's Health and Safety Services (HSS) office is responsible for ensuring the safety and security of all the high containment laboratories. The Keiller Building has electronic key card access. As of December, 2003, this building is guarded 24 hours a day, seven days a week, as required by the new federal regulations for buildings in which select agent research is conducted. Because of the high cost of security, UTMB has developed a plan to consolidate select agent research in only two buildings, Keiller and Pharmacology. A designated HSS staff member controls the issue of key cards and police undertake checks on select agent users as required by the USA Patriot Act.

We will update this policy in the future as required by law. Additions and deletions to the system are made through the University Police Department. Both systems track the employee through the UTMBI identification number, a unique number assigned to a new employee. The key card system identifies the ID number, date and time of entry into the building and biocontainment facilities. Removal of access can be accomplished within minutes should the need arise.

Encoded staff members have key card access at specific perimeter entry doors and to specific laboratories. An investigator and support staff are given access to only those laboratories approved for their work. All personnel have been finger printed and are registered with the CDC and the Department of Justice.

A senior Safety Coordinator-level HSS staff member is assigned to all the high containment laboratories and is on call at all times to respond to issues relating to safety and security. Both UTMB Facilities Operation & Management and HSS routinely conduct visual confirmation of performance several times per week. The frequency is increased according to project demands. Written authorization is required of any person entering the facilities that is not approved, and these individuals are kept at a minimum.

UTMB's HHS is a division of Preventive, Occupational & Environmental Medicine designed to maximize workplace health, safety and well being through nine integrated prevention programs. One component, the Biological and Chemical Safety Program has the mission of providing a comprehensive approach to health and safety for UTMB. The staff provides direction and consultation for the safe use, handling and storage of biological and chemical agents in the research, academic and healthcare arena. The program provides:

consultation and technical information for handling biological agents; reviews proposals and protocols for the use of hazardous biological agents, rDNA, and submits these to the Biological Safety Committee with recommendations; oversees the annual certification of biological safety cabinets by an outside contractor; presents biological safety seminars upon request; reviews and approves purchases of biological safety cabinets and other safety-related equipment; surveys laboratories for compliance with approved standards and policies of UTMB, CDC and NIH; provides assistance or advice in the disinfection of facilities and equipment; assists in the development of safety plans and training programs. Compliance The University of Texas Medical Branch has an existing safety program that is in accordance with appropriate Federal, State, and local regulations, and that hazards have been identified, eliminated, and/or controlled and that research can be performed safely under BSL1, BSL2, and BSL3 laboratory conditions. Similar safety programs are in development for the new BSL4 facilities that are under construction. All principal investigators are responsible for the implementation and adherence to University safety policies and procedures. Failure to do so could result in suspension or withdrawal of committee approvals for approved research projects.

Institutional BioSafety Committee UTMB researchers using BSL2 and BSL3 agents are required to have Institutional Biological Safety Committee (IBC) review and approval prior to use. The UTMB Biological Safety Committee requires the submittal of a Notification of Use for Biological Agents and rDNA (NOU) for biological agents meeting criteria for BSL2 and BSL3. Included in the NOU submittal are the written procedures for transport, surface decontamination, spill procedures, methods for disposal, methodology, transmission routes and a review of occupational health issues as well the description of Principal Investigators experience and scope of research. The IBC is directly linked into the University Employee Health Center and Health and Safety Services. The IBC meets once a month, allowing for timely review of the submitted NOU. Committee membership includes the County of Galveston Health Department Epidemiologist as a voting community member. CDC/HHS select biological agent users must have received Biological Safety Committee approval prior to CDC Select Agent Registration submission.

Training Additional training opportunities include Lab Safety Orientation, a 3-hour course covering laboratory safety issues in detail. Lab Safety Refresher is required every two years. EH&S staff are also available to develop and conduct topic specific training, as needed. Biosafety Level 3 users meet with Environmental Health and Safety prior to being allowed access to the BSL3 laboratory for initial BS L3 training. Users are referred to the Director of the BSL 3 facility to complete initial check of f to prepare for entry procedures. First time users are mentored during an initial training period then allowed limited key card access to the facility. Once training has been completed 24 hour, 7-day-a-week access is granted. Blood-borne pathogen training is conducted during initial employee orientation by UTMB Healthcare Epidemiology personnel and updated periodically. Radiation Safety training is provided by the Radiation Safety Program. Basic Radiation Safety in the Laboratory and Authorized User training programs and refreshers are provided to radioactive material users.

Disposal of Hazardous Materials Hazardous chemicals and radioactive materials are disposed and/or recycled by the Chemical and Radioactive material Pickup Program administered through Environmental Health and Safety, Environmental Protection Program. Chemicals are evaluated for potential recycling, reuse or final disposal. The University contracts with outside contractors for ultimate disposal of chemical and Radioactive wastes. Biohazardous waste is placed into red bag lined single use biohazard boxes. The boxes are transported to the campus Services Building and are incinerated on site. The University owns and maintains a licensed medical waste incinerator, which is operated by EnviroClean Inc. All disposable material leaving the BSL3 facilities are autoclaved, then placed in a biohazard container for incineration or non-disposable items are either autoclaved or chemically disinfected then sent out for final processing. Sharps containers are used to dispose of needles, capillary tubes, glass Pasteur pipettes and glass slides as required by the State of Texas Municipal Solid Waste Regulations for Healthcare Facilities.

The Center for Biodefense and Emerging Infectious Diseases (Keiller Building) BioSafety Level 3 Manual:

The Keiller BSL3 Manual is focused toward safety procedures and protocols for the *in vitro* BSL3 laboratory and the BSL3 insectary. The manual provides information such as entry procedures, spills, personal protective equipment, transportation of material and disinfectants.

EQUIPMENT

Baylor College of Medicine

The BCM laboratories are equipped with 3 New Brunswick BioFlo 3000 Fermentors, which have the capacity to run up to 10L fermentations (testing parameters including temperature, pH, dissolved oxygen, agitation rate, induction time, wet cell weight, and yield). This information is then transferred to scale up protein production. In addition, it contains a UV/VIS Spectrophotometer Smartspec 3000 (Biorad) Percival L33NLC8 intellus environmental incubator Vortex (2), stirrer (2), shakers, dry bath (2).

Equipment available for purification processes include: FPLC (2 *ÄKTA Explorer* and 1 *AKTA Pilot*), HPLC (Waters Liquid Chromatography System) or peristaltic pumps for larger scale processes. Other equipment and resources include: Victor3 multilabel counter (OPA), Invitrogen Xcell protein gel electrophoresis apparatus; SDS-PAGE-related equipment for protein characterization; Agarose and polyacrylamide gel electrophoresis equipment for analysis of nucleic acids and proteins; Balances, pH meter, water baths, microwave oven, and Milli-Q water system.

The imaging facility will include the following instrumentation:

- Siemens Inveon PET/SPECT/CT
- Bruker 9.4T/20 MRI
- Xenogen IVIS 100 Bioluminescence
- VisualSonics Vevo Ultrasound
- Siemens Sequoia ECHO
- RadSource RS 2000 Biological Irradiator

New York Blood Center

Equipment in Viral Immunology Laboratory

Tecan Ultra 384 multi-function reader; Beckman-Coulter Analytic Ultracentrifuge; Beckman L7-65 Ultracentrifuge; Carver tablet press; Tablet four usage tester; Osmometer (Model 3320, Advanced); *ÄKTA*TM purifier (FPLC) from GE; Waters HPLC System; Cytofluor 2300 fluorescence measurement system (Millipore); IBI STS45 DNA sequencing unit; Dynatech ML2252 Microtiter Luminescence Detection System; Spex Fluorolog FL212 Spectrofluorometer. Perkin Elmer Cetus Gene Amp PCR system 9600; 3 biosafety hoods; Pro/Pette liquid handling system (Perkin Elmer Cetus); Nikon fluorescence microscope; 3 microscopes, 2 inverted microscopes; DuPont Ommispin R; Sorvall RT 6000 and Sorvall RC-5C centrifuges; 5 freezers, 3 refrigerators, Isotec Fisher Laboratory Refrigerator; 3 CO₂ incubators; Coulter Electronics cell counter, 3 water baths; Oven; Labconco laboratory steam washer; Barnstead Nanopure system; Lab-line incubator-shaker; Savant Speed-Vac concentrator; 3 fraction collectors; Perkin Elmer spectrophotometer; Gilson Fluorometer; Biorad gel-dryer and slicer; Skatron 96-well cell harvester; Thermolyne Cryo Biological Storage System; Büchi Rotavapor R-124; The BSL3 facility has 2 biosafety hoods; an autoclave; Sorvall RC3, Beckman J-6B and Beckman L28-M. centrifuges; refrigerators and freezers.

Equipment in Laboratory of Molecular Parasitology

1 Leica 3MC dissecting scope, 2 Innova 4000 incubator shaker (New Brunswick Scientific Company), 1 Innova 44R incubator shaker (New Brunswick Scientific Company), 1 water bath; 1 Perkin-Elmer Cetus DNA thermal cycler, 1 Gene Machine II DNA thermal cycler, Equipment for gel electrophoresis and blotting, 1 dimensional and 2 dimensional, Sub Cell GT nucleic Acid electrophoresis systems, Prep Cell model 491 (Biorad), Rotofor Cell (Bio-Rad), 2 Laboratory Refrigerators (Precision 812 and Sears Coldspot), 1 Microplate Autowasher Dynex Utrawash Plus, 2 Imperial III incubators, 1 bench top centrifuge (Fisher), 2 Eppendorf Centrifuge 5415C and 4 microfuges, Uvicord SII, pump, fraction collector and recorder (Pharmacia Biotech), WL/Uv light box plus Polaroid Gel Cam camera, Microinjection apparatus, including a micromanipulator, A Horizontal needle puller PN-30, Nikon Eclipse E600 microscope with attached digital camera and video monitor, Electrophoresis power supplies (5), DigiTerm incubator (Tritech Research), UV/VIS Spectrophotometer Smartspec 3000 (Biorad),

Percival L33NLC8 intellus environmental incubator Vortex (2), stirrer (2), shakers, dry bath (2), 1 biological safety cabinet, 1 CO₂ water-jacketed incubator for tissue culture work (Forma Scientific), 1 Speed Vac Concentrator and a refrigerated vapor trap (Savant), 1 DrygelSr model SE 1160 (Hoefer Scientific), 1 Frost Free/Magic Chef Refrigerator, 2 -20°C freezers, So-Low Ultra Low and Cryo-Fridge, 2 vacuum pumps, 1 bench top Sorvall RT7 refrigerated centrifuge, 1 water bath, 1 Sorvall RC Plus centrifuge + SS-34 and SLA 3000 rotors, 1 Milli-Biocal and Milli-RiOs 8 water purification system (Millipore), Nikon Eclipse E400 microscope, Nikon TMS Tissue Culture Microscope, Eppendorf Centrifuge 5415C, Vortex, stirrer, pH meter, 2 balances, microwave, 1 Taylor Wharton K Series liquid nitrogen storage tank, 1 Revco ultra-low temperature freezer with 24-hour electronic temperature surveillance located in a central freezer facility, Zeiss Axioskop equipped with epifluorescence filter sets and digital camera.

Equipment located in the Core Facilities

Jasco circular dichroism (CD) spectropolarimeter (Model J-715, Jacobin, Japan); Biacore 3000 (Biacore, Piscataway, NJ); ; Electron Microscope, EM 410, Philips Electronic Instruments/FEI equipped with a Digital Camera; Zeiss LSM 510 META Confocal Microscope; Pi1er Peptide synthesizer (Applied Biosystems); Bench top alignment-free multipurpose analyzer from BD Immunocytometry Systems (FACSCanto); FACSCalibur analyzer; MoFlo high-speed cell sorter (DAKO Cytomation); Isothermal Titration Calorimetry (ITC); Differential scanning calorimetry (DSC); PEB Voyager DE Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometer; 492 Protein Sequencer; DNA Sequencer (3100 Genetic Analyzer); HPLC (HP 1100 & HP 1050); Sun Sparc Station I + computer workstation; 4 Packard Liquid Scintillation Counters: 3375, 3385, 2660, Minaxi 4430; 2 Nuclear Chicago Gamma Counters 1185, 1195; Viscometer (Model SVM 3000, Anton Paar); 3 Ludlum Survey Meters; Research Products International Series 900 Mini-Monitor; Packard Gamma Minaxi Counter; Shimadzu CS9000U Dual Wavelength Flying Spot Scanner; Tri-Carb 2900RT (Packard BioScience); Minaxi Auto-Gamma 5530 (Packard BioScience); Beta plate Flatbed Scanner 1205-001 (PerkinElmer); Storm 860 (Molecular Dynamics/Amersham Bioscience); Konica SRX-101A Film Developer; Alpha Beta Sample Counter

University of Texas Medical Branch

In Dr. Tseng's laboratory: 1 Microscope; Refrigerators; CO₂ incubators; Laminar flow hoods; Deep freezers; Refrigerated Centrifuges; Spectrophotometer; Photometer; Thermal cycler; Gel electrophoresis equipment

Flow Cytometry Unit - In the nearby MRB is the Flow Cytometry Unit for the UTMB campus. The unit has a Coulter EPICS Profile flow fluorocytometer, a Becton-Dickinson FACScan and a Becton-Dickinson FACSVantage flow cytometer.

PRODUCT DEVELOPMENT STRATEGY

Milestones and Timeline

Overview. The development of a vaccine represents a major endeavor taking years to achieve. It requires a program overseen by effective management that is based on a strong scientific evidence base, product development and manufacturing under a quality umbrella, and requisite preclinical and clinical studies. A Product Development Strategy (PDS) is generally drafted to provide an overall guidance, a set of specific and achievable goals, the schedules required to reach the ultimate objective, a set of alternative approaches, and the regulations that must be addressed, such as those related to product quality and safety.

This section, Milestones and Timeline, will describe the major development milestones including a risk management strategy if impediments arise that could require a revision in the work plan.

The likelihood of success for this project to develop an rRBD-based vaccine to prevent SARS relies on:

- 1) An extensive body of preliminary proof of concept data,
- 2) New innovations for product development and vaccine formulation,
- 3) The strong expertise of the operational management group, the technical units and industry partners with a track record of transitioning antigens through process development, cGMP manufacture, and FDA regulatory filings, and
- 4) Risk management strategies that include interim objectives, alternatives and accordingly, the decision points during the 5 years timeline.

One of the strength of this proposal is the selection of a group of experienced scientists from different fields that bring into this project the expertise to support all the activities within the proposal. The vaccine will be developed by BCM-Sabin, the Sabin Vaccine Institute's product development partnership located at Texas Children's Hospital and Baylor College of Medicine, in collaboration with academic partners at New York Blood Center (NYBC) and University of Texas Medical Branch Galveston (UTMB) and two industrial partners, Immune Design Corporation and Frontier BioSciences. In addition, we will collaborate through subcontracts with Walter Reed Army Institute of Research (WRAIR) for cGMP manufacture. A strength of the proposal is BCM-Sabin's 11 year track record of tech transfer of recombinant vaccines through cGMP manufacture with WRAIR including a hookworm vaccine that completed phase 1 clinical testing (42, 74, 75). BCM-Sabin will also provide regulatory and quality assurance and link our project with the industrial partners. Thus, experts have been specifically chosen for their individual strengths and our history of collaborating in other areas of vaccine product and clinical development.

The multi PD/PIs will be responsible for managing, coordinating, monitoring and supervising the many facets of the program to mitigate risk and maximize the likelihood for achieving the goals of each project, as well as to ensure that the activities interrelate and follow a strategic plan in the proposed timelines. Each collaborator will manage their activities and tasks at their respective sites and will be independently responsible for the hiring of personnel, the day-to-day supervision of projects, the recording of data according as required by the project, the recording of financial expenditure and keeping of accounts, the preparation of reports and ensuring regular interaction with other members of the consortium., including weekly teleconferences and/or meetings, in addition to quarterly program reviews with an expert advisory committee comprised of senior vaccine development experts.

Developmental Milestones. This R01 proposal aims to maximize success in the development of an anti-SARS vaccine based on the receptor binding domain (RBD) of the SARS-CoV spike (S) protein, by establishing the feasibility of upscale expression of the recombinant RBD (rRBD) protein, selecting the most appropriate expression system, and scalable manufacturing process. These activities include the development of a series of critical biochemical and biophysical assays for in-process, release and stability testing, which will result in a high-yield reproducible production process and a high quality and stable product that is potent and stimulates the desired protective antibody and cellular responses with co-formulated adjuvants. These represent essential steps for downstream pilot cGMP manufacture, GLP toxicology testing, IND filing with the U.S. FDA and ultimately clinical testing using adjuvant/vaccine combinations of choice. Ultimately the proposed studies will accelerate the development of the rRBD-based vaccine for human use.

To rapidly translate our initial proof of concept findings on the rRBD-based vaccine into a solid platform that will yield new approaches and strategies for product development, vaccine formulations will be optimized and

tested in mice to establish dose, schedule, and routes as well as confirmatory efficacy of the optimally expressed rRBD proteins *in vitro* and *in vivo* using two animal models. Since vaccination with the rRBD protein alone may not induce strong immune responses, alum based adjuvants as well as a novel TLR4 immunostimulant, GLA, will be assessed alone or in combination.

The **Ultimate Goal** of the proposed studies is to develop a highly effective and safe RBD-based vaccine that can be used in humans. SARS outbreaks remain a serious global concern mainly due to possible zoonotic reintroduction of SARS-CoV into humans or accidental release from a laboratory or deliberate spreading of the virus by a bioterrorist.

The proposal has three specific aims that will be performed in part in parallel with one another, while some activities will depend on the completion of the product development aspects of the project: 1) Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate; 2) Process development, characterization, formulation and stability profiling 3) Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. Ultimately, these studies will provide the basis for accelerating the conduct of a "first-in-human" Phase 1 study of the rRBD-based vaccine for preventing future SARS outbreaks and for biodefense preparedness.

The following milestones will be achieved through the performance and development of the specific aims detailed in this application:

Milestone 1. A suitable expression system is selected for expression of rRBD in small scale. Aim 1 will focus on the selection of the expression system expression based on yields, purity, antigenicity, functionality, immunogenicity, and efficacy (for inducing neutralizing antibody responses and protection against SARS-CoV challenge) of the rRBD protein. These attributes will be evaluated with qualified assays outlined in Table I. Based on the outcomes of assays each expression system will be scored and ranked as described in the proposal. An rRBD antigen preparation with a category score < 3 or final score < 15 will be removed from further consideration (see Table 1 in the proposal). A lead rRBD from one of the expression vectors with the highest final score will be selected for subsequent studies for optimization of immunization regimens (Aims 1E-G), and process development and pilot cGMP manufacture (Aims 2 and 3).

Table I. Assays for yield, purity, antigenicity, functionality, immunogenicity, and efficacy of rRBD protein

Application	Type of Assay
Yields	HPLC (RP-HPLC and/or SE-HPLC), protein concentration, SDS-PAGE stained with Coomassie Brilliant Blue or Silver Stain
Purity	HPLC and SDS-PAGE stained with Coomassie Brilliant Blue or Silver Stain, HPLC, Mass Spectrometry, Residual Host Proteins (by slot-blot)
Antigenicity	ELISA using RBD-specific and confirmation-dependent mAbs, including 33G4 (Conf V), 24H8 (Conf I), 31H12 (Conf II), and 35B5 (Conf IV), and human convalescent sera from at least three different SARS patients containing neutralizing antibodies.
Functionality	ELISA or ITC for measuring binding of rRBD protein to soluble ACE2 or flow cytometry for detecting the binding of rRBD protein to ACE2-expressing cells.
Immunogenicity	1) ELISA for determining the titers of the RBD-specific antibodies, including IgG1, IgG2a, IgG2b, IgG3 and IgA; 2) ELISPOT for MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte (CTL) responses, including the induction of IFN- γ , IL-2, IL-4 and IL-10.
Efficacy	1) Luciferase assays for measuring the titers of neutralizing antibodies against pseudoviruses expressing SARS-CoV S protein; 2) Cytopathic effect assay for determining the titers of neutralizing antibodies against live SARS-CoV infection; 3) use of the lethal MA15/BALB/c and/or SARS-CoV/ACE2 transgenic mouse models for evaluating the protection of rRBD-based vaccine against SARS-CoV challenge.

Milestone 2. Optimized immunization regimens that provide the best antigenicity, immunogenicity and efficacy for rRBD formulated with alum alone and/or in the presence of GLA, an innate immunostimulant. Once the most optimal expression system is selected, it will be used to generate research lots of rRBD protein for further characterization and confirmatory studies as well as for optimization of immunization regimens by testing different antigen doses and adjuvant formulations (Table II). Once the optimal dose and adjuvant formulation are identified, different routes of administration, number and interval of immunizations will be tested (Table III). The efficacy of the rRBD-based SARS vaccine to induce protection

and long-term immune responses will be evaluated in a SARS-CoV infection mouse model using an optimized immunization regimen (Table III) and challenge studies (Table IV). The goal is to find the best vaccine formulation and vaccination regimen that induces high titers of neutralizing antibodies and high levels of CTL responses as well as protection against homologous and heterologous virus challenge, while using the least amount of the rRBD antigen within the vaccine and number of immunizations.

Table II: Optimization of antigen doses and adjuvant formulation

Group	Treatment	Group	Treatment
1	rRBD (5 µg) + PBS, s.c., 3 immunizations	7	rRBD (10 µg) + GLA, s.c., 3 immunizations
2	rRBD (5 µg) + alum, s.c., 3 immunizations	8	rRBD (10 µg) + alum+GLA, s.c., 3 immunizations
3	rRBD (5 µg) + GLA, s.c., 3 immunizations	9	rRBD (20 µg) + PBS, s.c., 3 immunizations
4	rRBD (5 µg) + alum+GLA, s.c., 3 immunizations	10	rRBD (20 µg) + alum, s.c., 3 immunizations
5	rRBD (10 µg) + PBS, s.c., 3 immunizations	11	rRBD (20 µg) + GLA, s.c., 3 immunizations
6	rRBD (10 µg) + alum, s.c., 3 immunizations	12	rRBD (20 µg) + alum+GLA, s.c., 3 immunizations

Table III: Optimization of immunization route and dose frequency of immunizations

Group	Treatment	Group	Treatment
1	rRBD + adjuvant, s.c., 1 immunization	10	PBS + adjuvant, s.c., 2 immunizations, 4-week interval
2	PBS + adjuvant, s.c., 1 immunization	11	rRBD + adjuvant, i.m., 2 immunizations, 2-week interval
3	rRBD + adjuvant, i.m., 1 immunization	12	PBS + adjuvant, i.m., 2 immunizations, 2-week interval
4	PBS + adjuvant, i.m., 1 immunization	13	rRBD + adjuvant, i.m., 2 immunizations, 4-week interval
5	rRBD + adjuvant, i.d., 1 immunization	14	PBS + adjuvant, i.m., 2 immunizations, 4-week interval
6	PBS + adjuvant, i.d., 1 immunization	15	rRBD + adjuvant, i.d., 2 immunizations, 2-week interval
7	rRBD + adjuvant, s.c., 2 immunizations, 2-week interval	16	PBS + adjuvant, i.d., 2 immunizations, 2-week interval
8	PBS + adjuvant, s.c., 2 immunizations, 2-week interval	17	rRBD + adjuvant, i.d., 2 immunizations, 4-week interval
9	rRBD + adjuvant, s.c., 2 immunizations, 4-week interval	18	PBS + adjuvant, i.d., 2 immunizations, 4-week interval

Table IV: Efficacy of RBD-based vaccine to protect against SARS-CoV challenge in the lethal MA-15/Balb/c mouse model

Group	Treatment
1	Challenge mice with SARS-CoV 5 days post-last immunization, monitor the accumulated mortality, and sacrifice 3 survivals (if any) at day 3 rd , 6 th , and 9 th post-infection
2	Challenge mice with SARS-CoV 10 days post-last immunization, monitor the accumulated mortality, and sacrifice 3 survivals (if any) at day 3 rd , 6 th , and 9 th post-infection
3	Challenge mice with SARS-CoV 15 days post-last immunization, monitor the accumulated mortality, and sacrifice 3 survivals (if any) at day 3 rd , 6 th , and 9 th post-infection

Milestone 3. Established analytical/biochemical and biophysical assays for rRBD. Process development and downstream manufacturing rely on developing assays for the product's characterization (appearance, identity, conformation, integrity and purity), identification of contaminants, and evaluation of stability. The optimization and development of antigen-specific assays such as those shown in Table I will lead to the qualifications for each assay (sensitivity, specificity, accuracy, and reproducibility) as well as product specifications, (qualitative and quantitative ranges of values) which are essential quality and consistency criteria during the production process. Therefore, it is planned to simultaneously use and evaluate these assays as the recombinant rRBD is made available. Biologic products may be especially susceptible to degradation and potential biochemical modifications. These assays will be able to monitor the proteins and provide meaningful information on the stability of the recombinant proteins. In addition, various denaturing/solubilizing agents will be evaluated using the biophysical techniques. The goal will be to complete a phase diagram. The information obtained from these assays will identify potential stabilizers for the recombinant protein with the goal to maximize multiple aspects of physical stability.

TABLE V contains a tool-box of qualified assays, combined with those described in Table I, which will be used to assess the identity, integrity, bioactivity and stability of recombinant proteins during in-process, release and stability testing for rRBD-based SARS vaccine; all of which will support the success of finding the optimized conditions for the production of a scalable manufacturing process development for potent rRBD to support downstream pilot cGMP manufacture, toxicology testing, IND filing with the FDA and clinical testing.

Table V. Qualified analytical/biochemical and biophysical assays for assess the identity, integrity, stability of rRBD proteins during in-process, release and stability testing

(b)(4); (b)(3); 7 U.S.C. § 8401

Milestone 4. Established a reproducible 10L scale process for a stable rRBD-based vaccine in preparation for future technology transfer to a cGMP manufacturing facility. Biologics must be developed, produced and tested using a comprehensive framework that must consider a number of unique features related to yield and cost, purity, consistency, identity, antigenicity and immunogenicity, formulation, and thermostability. Under this aim, fermentation, purification and buffer formulation parameters will be further optimized and evaluated at the 10L scale. The goal will be to establish reproducible high-yield production processes. As these studies are completed, the stability of the rRBD will be assessed. Both accelerated and long term stability studies will be conducted on the final formulations by monitoring physical changes in the protein formulation over time as detected using biophysical assays. This activity is critical for the future success during technology transfer to a manufacturing facility. Based on the experiences of BCM-Sabin all the reagents, processes and equipment used at the 10L scale should be compatible to those to be used during the manufacturing process. Therefore, efforts will be made so that at the time of transfer, scale will be the only variable that could be modified, since changes in almost any other parameter could result in run failure.

Milestone 5. Successful pilot cGMP manufacture and completed GLP toxicology testing. Process development and formulation technology for the selected rRBD-based vaccine will be successfully transferred to the WRAIR pilot cGMP facility and both drug substance and drug product will be evaluated during the in process and final lot release. Certificate of Analyses (CoA) for the cell banks, drug substance and product will be generated and a formal stability program will be initiated. A pre-IND meeting will be requested with the U.S. FDA and GLP toxicology testing at Frontier Biosciences will be completed with an acceptable safety profile. The extensive array of assays developed and qualified throughout Aims 1 and 2 will be used to establish criteria for the evaluation of the expression during manufacturing and vaccine formulations. These same specifications will be used throughout the stability program.

Milestone 6. IND Regulatory Package Preparation and Submission. An IND package will be prepared containing all the information related to the chemical, manufacture and control (CMC) as well as results of all the preclinical animal studies, GLP toxicology results and a proposed clinical development plan. Similar to other vaccine development programs of BCM-Sabin, the ultimate intention is to have a completed IND package in order to advance into clinical testing and accelerate the "first-in-human" Phase 1 safety study of the rRBD-based vaccine for preventing future SARS outbreaks and for biodefense preparedness.

Risk management and alternative approaches that will support the above milestones are detailed within the proposal. As part of our risk management strategy, we build in alternative pathways to arrive at each milestone. For example in milestone 1, multiple expression systems are available to us both for *E. coli*

(including pET/BL21 systems, pTrcHis/BL21, and pBAD) and *P. pastoris* and *Saccharomyces*. For milestone 2 we have built in multiple immunization regimens, while for milestone 3 our system of assay development, qualification, and implementation has already led to two IND submissions with CMC section acceptable to the FDA. For milestone 4 through DOE and multiple process development runs we will maximize the likelihood of deriving a process to produce recombinant RBD at the highest yield and purity, while for milestone 5, we have an extensive track record of successful technology transfer to WRAIR, but we have also worked successfully with additional CMOs including Aeras Global TB Foundation, and our long-standing collaboration with Instituto Butantan, Brazil's major vaccine manufacturer. For milestone 6, we have a strong regulatory group, which has now successfully completed two INDs for neglected disease recombinant vaccines.

Invention report, patent status or other protection of project intellectual property (IP)

We have had the following patent related to the proposed studies: Jiang, S., and He, Y. Neutralizing monoclonal antibodies against severe acute respiratory syndrome-associated coronavirus. US Patent 7,629,443. Filed February 8, 2006. Issued December 8, 2009, assigned to the New York Blood Center. This invention shows that a recombinant protein containing the receptor-binding domain (RBD) in the SARS-CoV S protein (residues 318–510) could induce highly potent neutralizing antibody responses in the immunized mice, and thus a panel of 23 monoclonal antibodies (mAbs) with potent neutralizing activity and ability to recognize six different conformational epitopes in the RBD domain were discovered. Accordingly, the RBD of SARS S protein is an important target site for developing vaccines and immunotherapeutics. It is anticipated that BCM-Sabin will file an additional patent on the composition and use of the formulated rRBD SARS CoV vaccine.

TABLE VI. Proposed Project Timeline

	Year 1	Year 2	Year 3	Year 4	Year 5	Responsible Group
<i>Specific Aim 1. Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate</i>						
1.A. Feasibility of expression						BCM-Sabin
1.B. Antigenicity and functionality						NYBC
1.C. Immunogenicity						NYBC
1.D. Efficacy						NYBC/UTMB
1.E. Optimization of the immunization regimens						NYBC/UTMB
1.F. Efficacy of rRBD-based vaccine to induce cross-neutralizing antibody responses and cross-protection in mice						NYBC/UTMB
1G. Efficacy of the rRBD-based vaccine to induce long-term immune responses and protection.						NYBC/UTMB
<i>Specific Aim 2. Process development, characterization, formulation and stability profiling</i>						
2.A. Development and optimization of a 10L scale process (fermentation & purification)						BCM-Sabin
2.B. Assay development						BCM-Sabin
2.C. Execution of 3 successive process development runs at the 10L scale						BCM-Sabin/NYBC
2.D. Formulation and Stabilization						BCM-Sabin/NYBC
<i>Specific Aim 3. Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation</i>						
3.A. Strategy for Manufacture of Drug Substance and Drug Product						BCM-Sabin
3.B. Lot release and start of a stability program						BCM-Sabin
3.C. IND Regulatory Package Preparation and Submission						BCM-Sabin

Product Development Plan

Overview. The studies here will lead to the expression, process development, formulation, technology transfer, cGMP manufacture, GLP toxicology and preparation of a regulatory filing (IND submission) of a recombinant vaccine to prevent severe acute respiratory distress syndrome (SARS) caused by the SARS-associated coronavirus (SARS-CoV). The vaccine is comprised of the receptor binding domain (RBD) of the SARS-CoV S protein, expressed as a recombinant protein (rRBD), and formulated with alum and/or GLA, a innate immunostimulant. The studies here will accelerate the development and lead to the first Phase 1 clinical study of a recombinant anti-SARS-CoV vaccine. The vaccine will be developed by BCM-Sabin, the product development partnership of the Sabin Vaccine Institute located at the Feigin Center of Texas Children's Hospital Center and Baylor College of Medicine. BCM-Sabin has an eleven year track record of taking recombinant antigens for two neglected diseases, hookworm infection and schistosomiasis, from antigen discovery, through scale-up process development, formulation, cGMP, regulatory filing, and phase 1 clinical testing (2,20,42,44,67). To achieve the technological and product development objectives of this proposal, BCM-Sabin will collaborate with two academic partners at New York Blood Center (NYBC) and University of Texas Medical Branch Galveston (UTMB). NYBC has a seven year track record of discovering the rRBD antigen and accumulating preliminary data for its protection in experimental challenge models of SARS-CoV, while UTMB has developed an innovative transgenic mouse model that replicates human SARS. In addition two key industrial partners will be actively involved in the project: Immune Design Corporation and Frontier BioSciences as well as the Walter Reed Army Institute of Research (WRAIR) for cGMP manufacture.

Intended use / Indication of the product. The rRBD-based vaccine is a recombinant protein-based vaccine formulated with alum (either Alhydrogel® or aluminum phosphate) and/or GLA, a synthetic TLR4 (toll-like receptor 4) agonist known to induce potent antibody and cellular responses to by stander recombinant proteins (28). The initial indication for the vaccine is for adults and individuals over the age of 15 who are considered at greatest risk of mortality from SARS (68). The vaccine would be administered as an injectable product to protect against lethal SARS-CoV infection and as a single dose or two doses spaced closely together (within 2-4 weeks) so that vulnerable populations could be rapidly immunized in an outbreak setting (Table 1). Another aspect of the target product profile of the rRBD protein is that it would be highly stable (up to four years at 2-8°C) so that it can be stockpiled for emergency use. Finally, low-cost is desirable so that the vaccine could also be used in low- and middle-income country settings in Southeast Asia (where populations are at the greatest risk of acquiring SARS) as well as in the United States and Canada.

Table 1. Target Product Profile of the Recombinant RBD-based Vaccine.

Item	Desired Target
Indication	A preventative vaccine to protect against lethal infection caused by the SARS-CoV
Target Population	Adults and children ≥ 15 years
Route of Administration	Intramuscular injection
Product Presentation	Single-dose vials. 1.0 ml volume of delivery
Dosage Schedule	Maximum of 2 immunizations regardless of age, with the second injection given 2-4 weeks after the first immunization
Warnings and Precautions/Pregnancy and Lactation	Mild to moderate local injection site reactions such as erythema, edema and pain, the character, frequency, and severity of which is similar to licensed recombinant protein vaccines. Less than 0.01% risk of urticaria and other systemic allergic reactions. Incidence of SAEs no more than licensed comparator vaccines.
Expected Efficacy	80% efficacy at preventing SARS associated deaths
Co-administration	All doses may be co-administered with antiviral drugs and/or other vaccines used in public health emergencies
Shelf-Life	4 years
Storage	Refrigeration between 2 to 8 degrees Celsius. Cannot be frozen. Can be out of refrigeration (at temperatures up to 25 degrees) for up to 72 hours
Product Registration	Licensure by the U.S. Food and Drug Administration
Target Price	Less than \$10 per dose for use in low- and middle-income country settings

Addressing the biodefense / public health gap. The SARS-CoV is classified by NIAID-NIH as a Category C pathogen. SARS is considered the first new infectious disease of this new century, having originated in Guangdong province of China in November 2002 and ultimately spreading along international air-travel routes to and across five continents and leading to over 8,000 infections and almost 800 deaths, before the outbreak was contained through an aggressive program of quarantine, patient isolation and travel restrictions (1). The majority of SARS patients to date have been adults with only a few cases in children less than 15 years of age (68). While the overall mortality rate from SARS during the 2002-03 pandemic was approximately 10%, the mortality among the elderly exceeded 50% (1). In April of 2003, a new coronavirus (CoV), SARS-CoV, was identified as the etiologic agent of this deadly human respiratory disease. Like other animal CoVs, SARS-CoV is an envelope virus with a single stranded, positive-sense RNA genome encoding a nonstructural replicase polyprotein and several structural proteins including a spike (S) and envelope (E) protein (1). SARS CoV is a zoonotic virus that resides in bats and other natural reservoirs, including palm civets as key intermediate hosts. The virus replicates primarily in airway epithelial cells of humans as well as many different zoonotic hosts. Since the original outbreak, subsequent outbreaks were reported in late 2003 and early 2004, with the latest one in Beijing and Anhui China that originated from accidental exposure to a laboratory virus isolate. SARS outbreaks remain a serious global concern because of possible zoonotic reintroduction of SARS CoV into humans, further escape of laboratory isolates or being used for bioterrorism. The movement of SARS-CoV into human populations suggests an urgent need to prepare vaccines for this emerging agent (68). There is a particular need for high-risk groups including the elderly, healthcare workers and laboratory personnel and for biodefense preparedness (68).

Scientific and technical value of the project. The proposed studies would lead to the development of the first recombinant vaccine for preventing human SARS-CoV infection. Key strengths the product development plan include a strong evidence base of preliminary data for the protective efficacy of the rRBD molecule accumulated by NYBC over the last seven years, and BCM-Sabin's eleven year track record of scale up process development, formulation and tech transfer for cGMP manufacture.

The key technology objectives are the development of a process for vaccine manufacture, utilizing an appropriate expression system selected based on small scale feasibility studies using primarily a bacterial expression system and with a parallel evaluation of the yeast system as back-up. Process development will emphasize inexpensive fermentation and purification methods to produce the rRBD protein antigen component of the vaccine with optimized yields and minimized costs. These activities would take place at BCM-Sabin with confirmatory preclinical testing at NYBC and UTMB. During scale up and while establishing reproducibility, Batch Production Records (BPRs) will be utilized to ensure that complete records are maintained, and to make certain that production conditions can be repeated with absolute accuracy. Before transferring technology to a cGMP facility, the final process will be repeated a minimum of three times at the 10 L scale, to assure that vaccine manufacture, from fermentation through formulation, reproducibly results in product of sufficient yield and quality. In addition, antigen/adjuvant formulation studies will be established both biochemically and by immunological assessment (potency). By performing a minimum 3 full runs with in process and final quality control, we will ensure the adequate planning of this transitional step and prevent a transfer of the technology that could otherwise lead to significant losses in yield or an improperly folded recombinant protein adjuvant. Preliminary accelerated and long term stability studies will be conducted on formulations by monitoring analytical/biochemical and biophysical changes in the protein and formulation over time. Studies of the chemical stability of the recombinant protein with an emphasis on oxidative and deamidation processes will also be conducted using high pH to induce deamidation and various oxidizing agents to produce oxidation. Isoelectric focusing studies will initially be used to detect deamidated as well as some oxidized species. In addition, accelerated stability will be assessed at different time points and after doing temperature incursion experiments (repeated freeze thaw cycles, and at different temperatures). Following formulation of the vaccine on alum and/or GLA in collaboration with the Immune Design Corporation, the technology would be transferred to Walter Reed Army Institute of Research (WRAIR) for cGMP manufacture. The final product would be subjected to a rigorous battery of assays for lot and product release at BCM-Sabin and WRAIR. An added strength of the proposal is BCM-Sabin's decade-long history of tech transfer to WRAIR for recombinant protein vaccines and its long standing collaboration with Dr. Steve Reed previously CEO of IDRI and now CEO of Immune Design Corporation. Following GLP toxicology testing at Frontier BioSciences, an IND regulatory filing

for clinical testing will be prepared and submitted to the U.S. FDA with BCM-Sabin as the sponsoring institution.

The major innovations for this product include:

- Selecting to use recombinant RBD rather than the full-length S-protein as the vaccine antigen in order to limit the possibilities of vaccine induced antibody enhancement (see below).
- Use of a novel combination of biochemical/analytical and biophysical tools to profile and maximize the long-term stability of the vaccine, which is an essential element for a vaccine that might require emergency stockpiling. Biophysical tools including circular dichroism, intrinsic and extrinsic fluorescence, light scattering, and differential scanning calorimetry have been used previously to evaluate vaccine stability (34,35). Collectively, the combination of assays during the development phases provides specific information concerning the physical state of the protein as a function of stresses (i.e temperature and pH fluctuations). Such data will be used to develop assays to screen and identify potential stabilizers such as sugars and other buffering agents, preventing degradation or aggregation of recombinant proteins as well as ensuring compatibility of buffering agents with the adjuvants to be used during vaccine formulations.
- Access to the TLR4 agonist and synthetic lipid A adjuvant known as GLA (glucopyrranosyl lipid A). This 1.7 kDa synthetic molecule with 6 acyl chains and a single phosphorylation site was developed by the Immune Design Corporation (Seattle, WA). In a study in mice with commercial Fluzone®, a killed influenza vaccine, chemically synthesized GLA was effective in enhancing IgG titers (both IgG 1 and IgG2a), both as an oil-water emulsion (squalene oil and surfactant), and as an aqueous formulation (GLA-AF) (3, 4). Key Th1 cytokines, IL-2 and gamma interferon, were also elevated in this study (13). Moreover, a oil-in-water emulsion of GLA with a malaria vaccine, GMZ2 adjuvanted by aluminum hydroxide, have been shown to enable the induction of the highest (a) vaccine-specific IgG2a and total IgG titers, (b) parasite-specific IFA titers, (c) levels of Type 1 cytokine responses (IFN- γ), and (d) number of longlived-plasma cells secreting antibodies, which are thought to be essential for the development of long-term protective immunity against clinical malaria (28). Preclinical study of the efficacy of rRBD-based vaccine will be studied in two innovative and complementary lethal mouse models: 1) MA15/BALB/c mouse model using a mouse-adapted MA15 virus for homologous virus challenge since it originated from SARS-CoV Urbani strain and can cause SARS-like disease and mortality in BALB/c mice; 2) GD03/Tg AC70 mouse model: Transgenic mice, Tg AC70, express the human angiotensin-converting enzyme 2 (ACE-2) virus receptor (the human SARS-CoV receptor). They are susceptible to infection by both homologous and heterologous SARS-CoV of the clinical isolates, and thus they can be used for evaluation of cross protection. Following infection of such transgenic mice with SARS-CoV, they develop weight loss and other clinical manifestations before reaching 100% mortality within 8 days after intranasal infection (63). The severity of the disease developed in the Tg AC70 mice makes them valuable for preclinical testing of SARS vaccines (63).

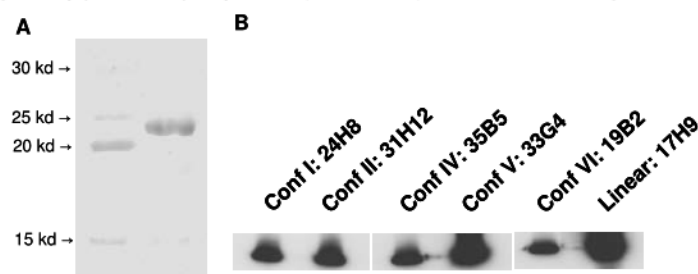
Advantages compared to competing products. Currently there are no approved vaccine and antiviral drugs that can be used for prevention and treatment of SARS-CoV infection (68). Several different strategies have been proposed for developing SARS vaccines, including inactivated virus vaccines, and the live attenuated vaccines, adenovirus vectored vaccines, poxvirus vectors, and DNA vaccines encoding the full-length spike (S) of SARS-CoV (68). Safety is the major concern regarding the inactivated SARS vaccines since the production workers are at risk of infection during their handling of the highly concentrated live SARS-CoV and incomplete inactivation may cause SARS outbreaks among vaccinated populations. The live attenuated or genetically engineered vaccines expressing full-length S protein of the 2002-2003 SARS-CoV may induce antibodies that enhance infection by civet SARS-CoV(69), which has been noted with feline CoVs (68,70,71). The ferrets vaccinated with a recombinant poxvirus vector MVA expressing SARS S protein displayed increased liver pathology after challenge compared to other groups (8,68), while vaccination with an N protein expressed in Venezuelan equine encephalitis virus replicon particles was reported to increase eosinophilic infiltration and damage in lungs of mice challenged with SARS-CoV (64). We are the first group that discovered that the RBD is the most important target for SARS vaccines since it contains the major neutralizing epitopes and some specific T cell epitopes, but no immunodominant non-neutralizing epitopes. Moreover, immunization of animals with the rRBD-based subunit vaccines could induce broad cross-neutralizing and protective activities against the predominant human SARS-CoV strains (9,10,29,40). Compared with the inactivated virus and live attenuated virus vaccines, the rRBD-based vaccine is safer since there is no risk to cause virus infection due to

the vaccination. It is also safer than the full-length S protein since the linear immunodominant domains in S protein could induce high titers of non-neutralizing antibodies (31), some of which could enhance infection by heterologous SARS-CoV, such as the civet SARS-CoV strains (32). Vaccination of ferrets with vaccinia virus-based SARS expressing full-length S protein also caused liver damage after challenge with SARS-CoV (7,33). So far, no one has shown that rRBD vaccines induce infection-enhancing antibodies or harmful immunity (1).

Project goals and key technological objectives. The major project goals to be completed during the 5 years award period include: 1) feasibility of expression evaluating both bacterial and yeast expression systems, and pre-clinical characterization of the differently expressed rRBD protein as a vaccine candidate; 2) rRBD and vaccine formulations will be compared for their ability to induce neutralizing antibodies and protection in laboratory animals against challenge infections with multiple strains of the SARS-CoV using an optimized vaccination regimen and adjuvant platforms; 3) development of a process for the high yield and low cost expression and purification of the RBD protein antigen, followed by formulation on alum (Alhydrogel® or aluminum phosphate) and/or an aqueous formulation with GLA, an TLR4 agonist; 4) development and qualification of product specific assays, followed by process and formulation optimization, which incorporates biophysical profiling; 5) Completing three process development pilot runs with in process and quality control testing; 6) technology transfer for the cGMP manufacture of master and production cell banks, drug substance and drug product; 7) formal release of the drug product based on qualified assays and initiation of a formal stability program; 8) a pre-IND meeting followed by completion of a GLP toxicology study, and 9) IND preparation and submission and approval for clinical testing. The major deliverable at the end of the project period is that the rRBD-based vaccine will be approved for Phase 1 clinical testing by the U.S. FDA. To date, BCM-Sabin, the product development partnership of the Sabin Vaccine Institute, has a track record of transitioning two recombinant parasite antigens for human hookworm infection all the way from discovery through process development, cGMP manufacture, and FDA approval for clinical testing (2,20,67). Another vaccine antigen for schistosomiasis is currently undergoing cGMP manufacture (2).

Performance specifications and features. In order to provide immunological benefit, the rRBD-based vaccine must elicit RBD-specific antibody and cytotoxic T lymphocyte (CTL) responses. In human infections, SARS CoV-specific IgG is detectable two weeks post-infection in humans reaching a peak approximately 60 days post-infection. High titers of neutralizing antibodies as well as specific CTL responses are detectable in patients who recover from SARS, with levels correlating with disease outcome. Neutralizing antibodies and/or CTLs from these individuals mainly target the S protein, specially the receptor-binding domain (RBD), which is comprised of 192 amino acids (amino acids 318-510) in the S1 subunit and is responsible for binding of the spike protein to the receptor ACE2. Indeed, we have found that more than 50% of the neutralizing activity in the sera of convalescent SARS patients is from anti-RBD specific antibody (10-12,30,39,40,72). Moreover, additional studies have shown that RBD can absorb and remove the majority of neutralizing antibodies in the antisera of rabbits, mice, and monkeys immunized with MVA expressing full length S protein (11). As noted below, RBD induces potent neutralizing antibody responses and long-term protective immunity in animal models, and RBD can induce potent cross-neutralizing antibodies against recombinant RBD from the SARS CoV of 2002-03 and 2003-04 human SARS-CoV isolates and the civet SARS CoV. The RBD of S protein contains multiple conformation-dependent epitopes and it is the main domain that induces neutralizing antibody and T-cell responses against SARS-CoV infection (1), making it an important vaccine target.

Description of the candidate product as currently configured. The rRBD-based vaccine is currently comprised of a purified recombinant RBD protein (expressed in *Escherichia coli*) and formulated on alum (either Alhydrogel® or aluminum phosphate) and/or an aqueous formulation of the synthetic TLR4 agonist glucopyrranosyl lipid A (GLA-AF). Shown in Fig. 1 is an rRBD protein expressed in pET-SUMO *E. coli* system



containing and purified by HPLC. The purified rRBD (without a tag) exhibits a single band with a molecular weight of 24 kDa on SDS-PAGE and reacts strongly with the RBD-specific and conformation-dependent neutralizing mAbs: 24HB (Conf I), 31H12 (Conf II), 35B5 (conf IV), 33G4 (Conf V) and 12B2 (Conf VI), as well as the linear epitope-specific mAb 17H9 in Western blot.

Fig. 1. Purification and identification of rRBD expressed in *E. coli*. (A) analysis of rRBD using SDS-PAGE and (B) identification of rRBD by Western blot using an panel of RBD-specific conformational and linear mAbs.

Assays for product release and characterization. Under a quality management system, BCM-Sabin has developed and implemented numerous assays for biochemical/immunological characterization. An existing panel of orthogonal techniques and assays are used in the earliest stages of process development, and then refined for the specific antigen concurrently with the program. These assays have provided important feedback to direct process development and formulation, regarding the state and stability of the molecule. Additionally, new assays and technology are typically identified and implemented where appropriate and to address specific characteristics of the molecule, such as multi-angle light scattering to detect protein aggregation. BCM-Sabin has performed similar assay qualifications to establish that the release and stability assays used for characterization are accurate, precise, sensitive, specific, reproducible, and robust. Several of these assays, currently used or being developed for RBD protein, have been qualified for other recombinant antigens by BCM-Sabin for Phase 1 clinical trials of hookworm vaccines. A description of the predominant assays proposed for characterization of the RBD protein antigen and formulation are presented below in Table 2. In addition, a variety of standard assays are developed and maintained to analyze pH, protein concentration determination by UV absorbance, and endotoxin content. These techniques will need be qualified and audited by BCM-Sabin's Quality Assurance unit prior to release testing of GMP lot.

Table 2. Qualified assays proposed for product release and characterization of the rRBD-based vaccine

Assay / (Application)	Description
<u>SE-HPLC</u> (bulk protein) In-process, Release, Stability	A size exclusion HPLC assay is used to analyze recombinant protein samples (crude fermentation supernatant to release of purified bulk). This assay has been developed for other antigens and adapted to RBD protein to quantitate target protein from other contaminants during in-process analysis. This assay also serves as a purity assay for RBD protein
<u>RP-HPLC</u> (Bulk protein) Release, Stability	Column and condition screening of a variety of RP-HPLC columns (including silica and polymer based) has been initiated to provide an assay that can better resolve full-length RBD protein from its product-derived cleaved forms (i.e. <1 kD difference).
<u>Multi-angle light scattering</u> (bulk protein) Information Only	A Wyatt Technologies multi-angle light scattering detector utilized both online (w/ SE-HPLC) and offline (Batch) has provided valuable data regarding the aggregation and native state of candidate antigens <i>IN SOLUTION</i> . This assay could be used to detect polydispersity of RBD protein and assist in stable formulation development
<u>SDS-PAGE</u> (Bulk protein & Formulation) In-Process, Release, Stability	SDS-PAGE assays under both non-reduced and reducing conditions allow determination of molecular weight, aggregation, and purity of samples. A calibrated scanning densitometer (Bio-Rad GS-800) allows a quantitative aspect to the assay, involving the determination of limit of detection and linear range of the assay to assess purity and relative quantity. This is assay, already adapted and implemented routinely for RBD protein analysis.
<u>N-Terminal Sequencing</u> (Bulk protein) Information Only	N-terminal sequencing has been completed on major, minor, and contaminant bands to identify whether contaminants are product derived and cleavage sites. SDS-PAGE is completed, transferred to a PVDF membrane and sent to a contracted service, audited by BCM-Sabin's Quality Assurance team and subject to a quality agreement.
<u>Mass Spectrometry</u> (Bulk protein) Information Only	Electrospray ionization mass spectrometry (ESMS) is carried out on a Micromass Q-ToF mass spectrometer on samples to monitor mass and any post-translational modifications. This assay will be used for RBD protein.
<u>O-phthalaldehyde (OPA)</u> (Formulation) Release, Stability	The OPA assay measures protein content by fluorogenic detection using o-phthalaldehyde (OPA), an amine-reacting reagent, in microplates. This technique is especially applicable to vaccine formulations where measurement of pellet (Alhydrogel®-bound) and supernatant (free-unbound) protein content is desired. This assay will be adapted to RBD protein and its formulation buffers and is widely used to determine binding efficiency.
<u>Host Cell Protein (HCP) Content</u> (Bulk protein) In-Process, Release	Residual host cell protein (HCP) impurities are analyzed using a slot blot system with a scanning calibrated densitometer with a picogram level of sensitivity in process fermentation and purification products and final purified protein samples.
<u>Potency, ELISA</u> (Formulation) Release, Stability	Potency will be determined by the median Effective Dose 50 (ED50), i.e., the minimal dose that seroconverts 50% of the mice vaccinated. Measurement of anti-RBD protein IgG will be performed by the indirect ELISA. We have applied this method successfully for the release of several hookworm antigens: it includes a robust in-house animal model and qualified ELISA procedures. A dose ranging study and generation of standard reference sera will be completed prior to assessing the performance characteristics specific to this antigen and qualification of RBD protein ELISA.

The following *in vitro* and *in vivo* assays will be used for evaluating the efficacy of the rRBD-based vaccine to induce specific antibody and T cell responses that protect animals from infection by homologous and heterologous SARS-CoV strains:

1. RBD-specific antibody and T cell responses. We will collect the sera of the rRBD-immunized mice for testing the titers of anti-RBD antibodies, including IgG1, IgG2a, IgG2b, IgG3 and IgA, using a well-established sandwich ELISA as we previously described (12,29,30,41). We will also collect the spleen and lymph nodes for assessing MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte response by using an ELISPOT mouse kit following *in vitro* priming with peptides bearing specific CD8 or CD4 epitopes that we identified (55,56).

2. Neutralizing antibody responses against pseudotyped and live SARS-CoV homologous and heterologous strains. We will determine the titers of the neutralizing antibodies in the sera of mice immunized with rRBD proteins using the pseudotyped viruses expressing the S protein of homologous and heterologous SARS-CoV strains Tor2, GD03, and SZ3 isolated from SARS patients at 2002-2003 and 2002-2003 and 2003-2004 outbreaks, and palm civet, respectively (Table 3)(39), as we previously described (12,29,30,41). We will also test the neutralizing antibody titers using live SARS-CoV Urbani (homologous) and GD03 (heterologous) strains as described before (30,40,55,57,63,64).

3. Viral replication in lung tissues of mice challenged with live SARS-CoV viruses. Mouse lung tissues will be collected for determining the titer of SARS-CoV (TCID₅₀/g of lung tissue) with the standard infectivity assay using Vero E6 cells or viral load (RNA copies/μg of lung tissues) using Q-RT-PCR as previously described (30,57,63,64).

We will use the following two animal models for evaluating the *in vivo* efficacy of the rRBD-based SARS vaccine to protect animals from infection by homologous and heterologous SARS-CoV infection: **1. The MA15/BALB/c mouse model.** This mouse SARS-CoV infection model was established by Roberts et al (62), who have shown that the mouse-adapted MA15 virus originated from SARS-CoV Urbani strain could infect BALB/c mice, resulting in SARS-like disease and high mortality (62). We will use this mouse model for evaluating the efficacy of rRBD to induce protection in the immunized animals against challenge with homologous SARS-CoV Urbani strain as previously described (62). **2. The icGD03-S/Tg AC70 mouse model.** This is the first transgenic mouse model of SARS-CoV infection established by Dr. Tseng's group at UTMB. We will use this model to evaluate the efficacy of rRBD-based vaccine against challenge with the homologous (Urbani) and heterologous (icGD03-S) SARS-CoV strains as previously described (57,63,64).

Rationale: An ideal vaccine for preventing SARS outbreaks must possess efficacy to induce high levels of neutralizing antibody response and protection in the vaccinated animals against a broad spectrum of SARS-CoV strains that may cause future outbreaks. We select both MA15/BALB/c and icGD03-S/Tg AC70 mouse models for evaluating the protective efficacy of RBD-based SARS vaccine because infection of SARS-CoV in these animal models can cause SARS-like disease and high mortality rate. Use of MA15/BALB/c mouse model is economic, but it cannot be used for evaluating the cross-protection against heterologous SARS-CoV challenge. To complement these studies, the icGD03-S/Tg AC70 mouse model can be used for evaluating the cross-protection against challenge with both homologous SARS-CoV strains, such as Urbani, and heterologous strains, e.g., icGDO3-S, which encodes the S glycoprotein gene of the most genetically divergent human strain, GDO3 (Table 3) (66) and could efficiently replicate in mouse lungs and in human airway epithelial cells (64). We will use the pseudotyped viruses expressing the S protein of the SARS-CoV strains Tor2, GD03, and SZ3, the representative strains of human 2002-2003 and 2003-2004 SARS-CoV and palm civet SARS-CoV, respectively (39), for evaluating the cross-neutralizing antibody responses in the rRBD-vaccinated mice because the future SARS outbreaks may be caused by a human SARS-CoV strain that may be accidentally released from a laboratory inventory or deliberately spread by a bioterrorist. The outbreak may also be caused by a new SARS-CoV isolate originated from the natural animal reservoir, like the palm civet SARS-CoV (73). We will use the intranasal route to challenge mice with SARS-CoV since this route was proven to be effective for SARS-CoV infection (55,64,74). The primary efficacy endpoint of rRBD-based SARS vaccine is the level of protection of the vaccinated animals against SARS-CoV challenge and the secondary endpoint is the ability of the rRBD protein to induce SARS-CoV neutralizing antibodies and specific T cell responses.

Table 3. Representative SARS-CoV strains that will be used for testing antibody neutralizing activities.

Virus	GenBank	Isolated from	Substitutes in	
	Accession#		S protein	RBD
Tor2	AY274119	SARS patients at 2002-2003 outbreak	0	0
Urbani	AY278741	SARS patients at 2002-2003 outbreak	0	0
MA15	DQ497008	Adapted from Urbani in BALB/c mice	1	1
GD03	AY525636	SARS patients at 2003-2004 epidemic	9	3
SZ3	AY304486	Palm civets	17	4

Regulatory experience and discussions with FDA. The vaccine will be developed by BCM-Sabin, Sabin's product development partnership (PDP) located at Baylor College of Medicine. BCM-Sabin is an established PDP for the development of vaccines to combat neglected diseases. BCM-Sabin serves as the regulatory sponsor for vaccines produced within the partnership, and has the lead role in filing INDs with the U.S. FDA and as well as other national regulatory authorities. BCM-Sabin submitted its first IND to the FDA in 2004, and conducted a Phase 1 clinical trial of the safety and immunogenicity of the *Na*-ASP-2 Hookworm Vaccine under BB-IND 12166 in the U.S (20,42,67). A second IND for a different hookworm vaccine has also been approved by the FDA and submitted to ANVISA for a phase 1 study in a hookworm-endemic area of Brazil in a field site established in collaboration with the Brazilian Ministry of Health's Fundação Oswaldo Cruz (2,44). In addition, a pre-IND meeting has been held for BCM-Sabin's new *Sm*-TSP-2 schistosomiasis vaccine. For this proposal, BCM-Sabin will review, synthesize, and assemble all preclinical data, CMC, toxicology data and a Phase 1 safety study protocol for the rRBD-based anti-SARS CoV vaccine and will submit an IND to the FDA.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:		* First Name:	Peter	Middle Name:	J
* Last Name:	Hotez	Suffix:	M.D.		
Position/Title:	Professor	Department:	Pediatrics		
Organization Name:	Baylor College of Medicine	Division:	College of Medicine		
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* E-Mail:	hotez@bcm.edu				
Credential, e.g., agency login:	eRA Commons User Name				
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Degree Type:	MD PhD				
Degree Year:	1978				
* Attach Biographical Sketch	1249-Biosketch_Hotez.pdf	Add Attachment	Delete Attachment	View Attachment	
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment	

PROFILE - Senior/Key Person 1

Prefix:		* First Name:	Shibo	Middle Name:	
* Last Name:	Jiang	Suffix:	M.D.		
Position/Title:	Member & Laboratory Head	Department:	Viral Immunology		
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Credential, e.g., agency login:	eRA Commons User Name				
* Project Role:	PD/PI	Other Project Role Category:			
Degree Type:	MD Ph.D				
Degree Year:	1978				
* Attach Biographical Sketch	1250-Biosketch_Shibo.pdf	Add Attachment	Delete Attachment	View Attachment	
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment	

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2			
Prefix:		* First Name:	Maria
		Middle Name:	Elena
* Last Name:	Bottazzi	Suffix:	Ph.D
Position/Title:	Professor	Department:	Pediatrics-Tropical Medicine
Organization Name:	Baylor College of Medicine	Division:	College of Medicine
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* E-Mail:	bottazzi@bcm.edu		
Credential, e.g., agency login:	eRA Commons User Name		
* Project Role:	PD/PI	Other Project Role Category:	
Degree Type:	Ph.D.		
Degree Year:	1995		
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Attach Current & Pending Support		Add Attachment	Delete Attachment View Attachment

PROFILE - Senior/Key Person 3			
Prefix:		* First Name:	Sara
		Middle Name:	
* Last Name:	Lustigman	Suffix:	Ph.D
Position/Title:	Member & Laboratory Head	Department:	Molecular Parasitology
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* State:	NY: New York	Province:	
* Country:	USA: UNITED STATES	* Zip / Postal Code:	10065-6275
* Phone Number:	212-570-3119	Fax Number:	
* E-Mail:	slustigman@nybloodcenter.org		
Credential, e.g., agency login:			
* Project Role:	Co-Investigator	Other Project Role Category:	
Degree Type:	Ph.D.		
Degree Year:	1985		
* Attach Biographical Sketch	1252-Biosketch_Lustigman.pdf	Add Attachment	Delete Attachment View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4			
Prefix:		* First Name:	Chien-Te
		Middle Name:	Kent
* Last Name:	Tseng	Suffix:	Ph.D
Position/Title:	Associate Professor	Department:	Microbiology and Immunology
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Credential, e.g., agency login:			
* Project Role:	Co-Investigator	Other Project Role Category:	
Degree Type:	Ph.D.		
Degree Year:	1997		
* Attach Biographical Sketch	1253-biosketch_Kent.pdf	Add Attachment	Delete Attachment View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment View Attachment

PROFILE - Senior/Key Person 5			
Prefix:		* First Name:	Lanying
		Middle Name:	
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* E-Mail:	ldu@nybloodcenter.org		
Credential, e.g., agency login:			
* Project Role:	Co-Investigator	Other Project Role Category:	
Degree Type:	Ph.D.		
Degree Year:	2007		
* Attach Biographical Sketch	1254-Biosketch_Lanying.pdf	Add Attachment	Delete Attachment View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 6			
Prefix:		* First Name:	Bin
		Middle Name:	
* Last Name:	Zhan	Suffix:	M.D.
Position/Title:	Professor	Department:	Pediatrics-Tropical Medicine
Organization Name:	Baylor College of Medicine	Division:	College of Medicine
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Street2:			
* City:	Houston	County/ Parish:	
* State:	TX: Texas	Province:	
* Country:	USA: UNITED STATES	* Zip / Postal Code:	77030-3411
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Credential, e.g., agency login:			
* Project Role:	Co-Investigator	Other Project Role Category:	
Degree Type:			
Degree Year:			
* Attach Biographical Sketch	1255-Zhan_biosketch.pdf	Add Attachment	Delete Attachment View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment View Attachment

PROFILE - Senior/Key Person 7			
Prefix:		* First Name:	Tehsheng
		Middle Name:	
* Last Name:	Chan	Suffix:	PhD
Position/Title:	Professor	Department:	Microbiology and Immunology
Organization Name:	University of Texas Medical Branch	Division:	School of Medicine
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Street2:			
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* E-Mail:	tchan@utmb.edu		
Credential, e.g., agency login:			
* Project Role:	Co-Investigator	Other Project Role Category:	
Degree Type:			
Degree Year:			
* Attach Biographical Sketch	1256-Biosketch_Chan.pdf	Add Attachment	Delete Attachment View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment View Attachment

BIOGRAPHICAL SKETCH

NAME Peter Hotez MD PhD		POSITION TITLE President, Sabin Vaccine Institute	
eRA COMMONS USER NAME eRA Commons User Name		Distinguished Research Professor and Chair Microbiology, Immunology, & Tropical Medicine	
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Yale University (magna cum laude)	BA	1976 - 1980	Molec. Biophysics
Rockefeller University	PhD	1980 - 1987	Biochemistry (Parasitol.)
Cornell University Medical College	MD	1980 - 1987	Medical Science
Harvard Medical School (Mass. Gen. Hosp)	Residency	1987 - 1989	Pediatrics
Yale University School of Medicine	Fellowship	1989 - 1991	Molecular Parasitology

A. Personal Statement.

Professor Peter Hotez is a laboratory and clinician investigator with a major interest in recombinant protein vaccines for neglected tropical diseases (NTDs). He is an elected member of the Institute of Medicine and founding director of one of the first non-profit public private partnerships (PDP) for developing vaccines to combat NTDs. PDPs use industrial practices but develop products in the non-profit sector because they target diseases of the world's poorest people. Launched in 2000, the PDP of the Sabin Vaccine Institute filed its first Investigational New Drug application for a recombinant vaccine in 2004, with a second IND filed this year. Today the Sabin PDP is also developing recombinant vaccines for schistosomiasis, and other NTDs. These vaccines are engineered in yeast and bacteria. Process development at the 10 liter fermentation scale, followed by protein purification and formulation studies are conducted at Sabin followed by technology transfer to a pilot scale manufacturer either in the U.S. or Brazil, and then clinical testing either in the U.S. or in rural areas of Brazil where the prevalence of these NTDs is close to or exceeds 50%. Sabin's PDP will relocate to Baylor College of Medicine on September 1, 2011 where it will be known as Sabin Vaccine Institute and Texas Children's Center for Vaccine Development.

B. Positions and Honors.

Positions and Employment

1991-92 Instructor, Pediatrics, Yale University
 1992-95 Assistant Professor, Pediatrics/Epidemiology & Public Health, Yale University
 1995-00 Associate Professor, Epidemiology & Public Health/Pediatrics, Yale University
 2000-11 Professor and Chair, Department of Microbiology, Immunology and Tropical Medicine, The George Washington University
 2006-11 Walter G. Ross Professor of Basic Science Research, The George Washington University
 2008-11 Distinguished Research Professor, The George Washington University
 2007- President, Sabin Vaccine Institute
 2007- Editor-in-Chief, *PLoS Neglected Tropical Diseases*
 2010- President, American Society of Tropical Medicine and Hygiene
 2011- Professor and Head of Pediatric Tropical Medicine, Baylor College of Medicine (after 9-1-11)
 2011- Dean, National School of Tropical Medicine at Baylor College of Medicine (after 9-1-11)

Honors (after 2005)

2006 Leverhulme Medal, Liverpool School of Tropical Medicine
 2006 Ambassador, Paul G. Rogers Society for Global Health Research, ResearchAmerica!
 2007 Member of Advisory Board (Council), Fogarty International Center, National Institutes of Health

2008 Science and Technical Advisory Group on Neglected Tropical Diseases, WHO
 2008 Member, Institute of Medicine of the National Academies
 2009 Science and Technical Advisory Committee on Tropical Disease Research, WHO
 2011 Member, National Institutes of Health Council of Councils

C. Selected peer-reviewed publications (in chronological order). (Selected from 218)

1. Hotez PJ, Ferris M. The antipoverty vaccines. *Vaccine* 2006; 24: 5787-99.
<http://www.ncbi.nlm.nih.gov/pubmed/16759763>
2. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Ehrlich Sachs S, Sachs JD, Savioli L. Control of neglected tropical diseases. *N Engl J Med* 2007; 357: 1018-27.
<http://www.nejm.org/doi/full/10.1056/NEJMra064142>
3. Loukas A, Bethony J, Brooker S, Hotez PJ. Hookworm vaccines – past, present and future. *Lancet Infect Dis* 2006; 6: 733-41. <http://www.ncbi.nlm.nih.gov/pubmed/17067922>
4. Diemert DJ, Bethony JM, Hotez PJ. Hookworm vaccines. *Clin Infect Dis* 2008; 46: 282-8.
<http://ukpmc.ac.uk/abstract/MED/18171264>
5. Hotez PJ, Brindley P, Bethony J, King CH, Pearce E, Jacobson J. Helminth infections: the great neglected tropical diseases. *J Clin Invest* 2008; 118: 1311-21. PMID: PMC2276811
6. Hotez PJ, Bethony J, Costa Oliveira S, Brindley PJ, Loukas A. A multivalent anthelmintic vaccine to prevent hookworm and schistosomiasis. *Expert Rev Vaccines* 2008; 7: 745-52.
<http://www.ncbi.nlm.nih.gov/pubmed/18665774>
7. Bethony JM, Simon G, Diemert DJ, Parenti D, Desrosiers A, Schuck S, Fujiwara R, Santiago H, Hotez PJ. Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 hookworm vaccine in unexposed adults. *Vaccine* 2008; 26: 2408-17. <http://www.ncbi.nlm.nih.gov/pubmed/18396361>
8. Hotez PJ. A plan to defeat neglected tropical diseases. *Sci Am* 2010; 302: 90-6.
<http://www.ncbi.nlm.nih.gov/pubmed/20063641>
9. Hotez PJ, Fenwick A, Savioli L, Molyneux DH. Rescuing the “bottom billion” through neglected tropical disease control. *Lancet* 2009; 373: 1570-4. <http://www.ncbi.nlm.nih.gov/pubmed/19410718>
10. Pearson MS, Bethony JM, Pickering DA, de Oliveira LM, Jariwala A, Santiago H, Miles AP, Zhan B, Jiang D, Ranjit N, Mulvenna J, Tribolet L, Plieskatt J, Smith T, Bottazzi ME, Jones K, Keegan B, Hotez PJ, Loukas A. An enzymatically inactivated hemoglobinase from *Necator americanus* induces neutralizing antibodies against multiple hookworm species and protects dogs against heterologous hookworm infection. *FASEB J* 2009; 23: 3007-19. PMID: PMC2735369
11. Hotez PJ. Peace through vaccine diplomacy. *Science* 2010; 327: 1301. PMID : 20223952.
<http://www.sciencemag.org/content/327/5971/1301.summary>
12. Pearson MS, Pickering DA, Tribolet L, Cooper L, Mulvenna J, Oliveira LM, Bethony JM, Hotez PJ, Loukas A. Neutralizing antibodies to the hookworm hemoglobinase Na-APR-1: implications for a multivalent vaccine against hookworm infection and schistosomiasis. *J Infect Dis* 2010; 201:1561-9. PMID 20367477.
<http://jid.oxfordjournals.org/content/201/10/1561.short>
13. Zhan B, Perally S, Brophy PM, Xue J, Goud G, Liu S, Deumic V, de Oliveira LM, Bethony J, Bottazzi ME, Jiang D, Gillespie P, Xiao SH, Gupta R, Loukas A, Ranjit N, Lustigman S, Oksov Y, Hotez P. Molecular Cloning, Biochemical Characterization and Partial Protective Immunity of the Heme-binding Glutathione S-Transferases from the Human Hookworm *Necator americanus*. *Infect Immun* 2010; 78:1552-63. PMID: PMC2849424
14. Hotez PJ, Pecoul B. “Manifesto” for the control and elimination of the neglected tropical diseases. *PLoS Neglect Trop Dis* 2010; 4: e718. PMID: PMC2876053
15. Hotez PJ, Bethony JM, Diemert DJ, Pearson M, Loukas A. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat Rev Microbiol* 2010; 8: 814-26. PMID: 20948553
<http://www.nature.com/nrmicro/journal/v8/n11/abs/nrmicro2438.html>

D. Research Support.

Ongoing Research Support

32472 Hotez (PI) 08/01/2000 - 07/31/2011

Human Hookworm Vaccine Initiative 1

Sabin Vaccine Institute is conduit for The Bill & Melinda Gates Foundation

Human Hookworm Vaccine Initiative (HHVI): Clinical Development & Evaluation of the *Na*-ASP-2 Hookworm Vaccine

The goal of this study is to continue product development, including the manufacture of a second pilot lot, and to conduct a global health impact analysis of the human hookworm vaccine with the Sabin Vaccine Institute

38988 Hotez (PI) 08/01/2006 - 07/31/2011

Human Hookworm Vaccine Initiative 2

Sabin Vaccine Institute-Human Hookworm Vaccine Initiative (HHVI): To develop and test the *Na*-APR-1 Hookworm Vaccine

The goal of this study is to conduct the process development, cGMP manufacture and testing, and clinical evaluation of APR-1 in order to develop a bivalent human hookworm vaccine with the Sabin Vaccine Institute

RCA8608 Hotez (PI) 09/30/2009 - 09/30/2011 NCE

(b)(4) -PATH-MVI

Development of a Malaria transmission blocking Vaccine

To develop a feasibility of expression study for the expression and scale-up of the *Anopheles*-APN-1 target candidate antigen as a malaria transmission blocking vaccine. The goal of this study is to conduct the early feasibility of expression using both yeast and bacterial expression systems.

1UL1RR031988-01 Hotez (GW PI) 07/01/2010 – 06/30/2015

National Institutes of Health

Clinical and Translational Science Institute at Children's National (CTSA)

The goal of the project is to provide highly integrated, cost effective, investigator-focused resources designed to overcome obstacles to investigation, promote collaborative research, and be continuously monitored and evaluated to identify when further adaptation or modification is needed.

Hotez (PI) 01/01/2011-12/31/2014

Dutch Government

Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the *Na*-GST-1 and *Na*-APR-1 hookworm antigens in both adults and children.

AI 90577 Hotez (PI) 01/01/2011 – 12/31/2012

National Institutes of Health

Title: Product development of a membrane tetraspanin vaccine against schistosomiasis

The goal of this project is the development of a high-yield, low-cost process for producing and formulating a recombinant Sm-TSP-2 schistosomiasis vaccine (10-liter scale)

Bottazzi/Hotez (MPI) 09/01/2010 - 08/31/2013

Instituto Carlos Slim de la Salud

Slim Initiative for Antipoverty Vaccine Development

The main goal of this project is to build a new generation of urgently needed vaccines for the neglected diseases, and to build capacity for vaccine development in Mexico.

Hotez (PI) 09/01/2011 – 07/31/2016

(b)(4)

Human Hookworm Vaccine Initiative 3

Clinical Development and Evaluation of the Na-GST-1 and Na-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Completed Research Support (selected support after 2000)

Clinical Research Grant Hotez (PI) 2000 - 2003

Private Source

Cloning and vaccine testing of novel hookworm antigens based on human serologic responses to identify human correlates of immunity against hookworm vaccine antigens in China

Role: Principal Investigator

Parasitology grant 98-674 Wang (PI) 1998 - 2001

China Medical Board of New York

Goals to develop DNA vaccines and diagnostic reagents for malaria, trichinellosis and hookworm and to establish Beijing as a world-class center of molecular parasitology. Role of Dr. Hotez: To establish a China Medical Board Scholars Program between Yale, GW, Peking Union Medical College, Capital University of Medical Sciences, and the Institute of Parasitic Diseases.

Role: Co-Investigator

RO1 AI-32726-09 Hotez (PI) 1996 - 2004

NIAID-NIH

Hookworm Larval Infectivity & Development

The major goals are to understand the mechanism of protection afforded by vaccination with recombinant hookworm antigens

Role: Principal Investigator

5 U54 AI57168 Levine (PI) 09/04/2003 - 02/28/2009

NIH/NIAID

RCE-Biodefense & Emerging Infectious Diseases

GWU Administrative Core and Media Training for Biodefense Scientists

The goals are to provide biodefense scientists the tools to handle media contacts in the case of a biodefense emergency with training that uses educational methods to obtain successful media interviews by focusing on extensive message discipline, delivery, physical appearance, presentation and relaxation techniques.

Role: Co-PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jiang, Shibo	POSITION TITLE Member & Lab Head
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
The First Medical University of PLA, China	M.D.	1978	Medicine
The First Medical University of PLA, China	M.S.	1983	Immunology
The Fourth Medical University of PLA, China	Ph.D.	1995	Virology
The Rockefeller University, New York.	Post-doc.	1987-1990	Immunology

A. Personal Statement

The goal of the proposed research is to develop an effective and safe severe acute respiratory distress syndrome (SARS) vaccine based on the receptor binding domain (RBD) of the SARS spike (S) protein for preventing the SARS outbreak or biodefense preparedness, through the selection of the appropriate expressions system, establishment of scalable manufacturing process and cGMP manufacture, as well as the completion of GLP toxicology testing and preparation of a regulatory filing. I, as the PI#2, will be responsible for planning, directing and executing the majority of the studies in Aim 1. I have a broad background in immunology, virology, infectious diseases and biomedicine. As PI on several NIH grants, I have accumulated extensive experience in development of vaccines against SARS-CoV. Since 2004, I have authored and coauthored more than 30 peer-reviewed papers related to SARS and influenza vaccine studies. My team is the first group in the world to show that the RBD of SARS-CoV S protein contains multiple conformation-dependant neutralizing epitopes, and vaccination of animals with recombinant RBD resulted in highly potent neutralizing antibodies and protection against SARS-CoV challenge, suggesting a great potential to develop rRBD-based SARS vaccine. In my lab, a series of techniques have been established for expression of rRBD proteins and evaluation of their antigenicity, functionality and immunogenicity. Most of the facilities, instruments and reagents required for the proposed studies are available. Therefore, my expertise, experience and a strong record of publications and extramural funding will allow me to act as the PI#2 in this project and achieve the goal proposed.

B. Positions and Honors

Positions and Employment

1978-85 Teaching Instructor (1978-82) and Lecturer (1983-85), the 1st Medical University of PLA, China.
 1987-90 Postdoctoral Fellow, Laboratory of Cellular Physiology & Immunology, Rockefeller University, NY.
 1990- Assistant Member (1990-96), Associate Member (1997-06), Member (2006-), & Lab Head (2002-), Viral Immunology Laboratory, Lindsley F. Kimball Research Institute, New York Blood Center, NY.

Other Experience and Professional Memberships

1998 *Ad hoc* Member, NIID Special Emphasis Panel on Innovative AIDS Vaccines.
 2000- Member, International Society for Antiviral Research (ISAR) and International AIDS Society (IAS).
 2002 *Ad hoc* Member, NIAID Special Emphasis Panel on *In vitro* Test Systems for Evaluating Chemotherapies against AIDS.
 2004 *Ad hoc* Member, NIAID Special Emphasis Panel on Innovative Research Topic in Virology.
 2004 External Reviewer for Canadian Institutes of Health Research (CIHR).
 2004- Member, Overseas Assessor Board for Chinese Academy of Sciences.
 2005- External Reviewer for Chinese National Sciences Foundation (CNSF).
 2005- Editorial Consultant for *The Lancet*.

- 2007-09 Regular Member, AIDS Discovery & Development of Therapeutics (ADDT) Study Section of NIAID.
 2008- External Reviewer for Hong Kong Research Grant Council (RGC).
 2010 *Ad hoc* Member, NIAID Special Emphasis Panel on SARS-CoV-Host Cell Interactions & Vaccine Development.

Honors

- 1985 & 86 Research Excellence Award, the First Medical University of PLA.
 1985 Young Scientist Award, Guangzhou Youth Medical Worker Association.
 1987 World Health Organization (WHO) Fellowship
 1988 Cancer Research Institute Fellowship
 2003, 06, 09 New York Blood Center President Award for Special Recognition as Investigator in Research.

C. Selected Peer-reviewed Publications (Selected from 244 peer-reviewed publications)

Most relevant to the current application

1. He, Y., Lu, H., Siddiqui, P., Zhou, Y., and **Jiang, S.** (2005). Receptor-binding domain of SARS-CoV S protein contains multiple conformation-dependant epitopes that induce highly potent neutralizing antibodies. *J. Immunol.* 174, 4908-4915. <http://www.ncbi.nlm.nih.gov/pubmed/15814718>
2. He, Y., Li, J., Li, W., **Lustigman, S.**, Farzan, M., and **Jiang, S.** (2006). Cross-neutralization of human and palm civet SARS-CoV variants by antibodies targeting the receptor-binding domain of spike protein. *J. Immunol.* 176, 6085-6092. <http://www.ncbi.nlm.nih.gov/pubmed/16670317>
3. **Du, L.**, Zhao, G., He, Y., Guo, Y., Zheng, B.-J., **Jiang, S.**, and Zhou, Y.S., (2007). The receptor-binding domain of SARS coronavirus spike protein induces long-term protective immunity in an animal model. *Vaccine.* 25, 2832-2838. <http://www.ncbi.nlm.nih.gov/pubmed/17092615>
4. **Du, L.**, Zhao, G., Chan, C., Li, L., Sun, S., Liu, Z., Guo, H., He, Y., Zhou, Y., Zheng, B.J., **Jiang, S.** (2009). Recombinant receptor-binding domain of SARS-CoV spike protein expressed in mammalian, insect and E. coli cells elicits potent neutralizing antibody and protective immunity. *Virology.* 393, 144-150. PMC2753736
5. **Du, L.**, He, Y., Zhou, Y., Liu, S., Zheng, B., **Jiang, S.** (2009). The spike protein of SARS-CoV – a target for vaccine and therapeutic development. *Nature Rev. Microbiol.* 7, 226-236. PMC2750777

Additional recent publications of importance to the field (in chronological order)

1. He, Y., Zhou, Y., Liu, S., Kou, Z., Li, W., Farzan, M., and **Jiang, S.** (2004). Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. *Biochem. Biophys. Res. Commun.* 324, 773-781. <http://www.ncbi.nlm.nih.gov/pubmed/15474494>
2. He, Y., Zhou, Y., Wu, H., Luo, B., Chen, J., Li, W., and **Jiang, S.** (2004). Identification of immunodominant domains in the SARS-CoV S protein: implication for developing SARS diagnostics and vaccines. *J. Immunol.* 173, 4050-4057. <http://www.ncbi.nlm.nih.gov/pubmed/15356154>
3. He, Y., Zhou, Y., Siddiqui, P., and **Jiang, S.** (2004). Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry. *Biochem. Biophys. Res. Commun.* 325, 445-452. <http://www.ncbi.nlm.nih.gov/pubmed/15530413>
4. He, Y., Zhu, Q.Y., Liu, S., Zhou, Y.S., Yang, B., Li, J., and **Jiang, S.** (2005). Identification of a critical neutralization determinant of SARS-associated coronavirus: importance for designing SARS vaccines. *Virology* 334, 74-82. <http://www.ncbi.nlm.nih.gov/pubmed/15749124>
5. He, Y., Li, J., Heck, S., **Lustigman, S.**, **Jiang, S.** (2006). Antigenic and immunogenic characterization of recombinant baculovirus-expressed SARS-CoV S protein: implication for vaccine design. *J. Virol.* 80, 5757-5767. PMC1472569
6. He, Y., Li, J., Yan, X., Hu, G., Zhou, Y. and **Jiang, S.** (2006). Identification and characterization of novel neutralizing epitopes in the receptor-binding domain of SARS-CoV spike protein: revealing the critical

antigenic determinants in inactivated SARS-CoV vaccine. *Vaccine*. 24, 5498-5508.
http://www.ncbi.nlm.nih.gov/pubmed/16725238

7. **Du L**, Zhao G, Lin Y, Sui H, Chan C, Ma S, He Y, **Jiang S**, Wu CY, Yuen KY, Jin DY, Zhou Y, Zheng BJ. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. *J Immunol*. 2008;180(2):948-956. PMC2603051
8. **Du, L.**, Zhao, G., Lin, Y., Chan, C., He, Y., **Jiang, S.**, Wu, C. Jing, D., Yuen, K.Y., Zhou, Y., Zheng, B.J. (2008). Priming with rAAV encoding RBD of SARS-CoV S protein and boosting with RBD-specific peptides for T cell epitopes increase T cell responses and provide protection against SRAS-CoV infection. *Vaccine*. 26, 1644-1651. PMC2600875
9. **Du, L.**, Zhao, G., Li, L., He,Y., Zhou,Y., Zheng, B.J., **Jiang, S.** (2009). Antigenicity & immunogenicity of SARS-CoV S protein receptor-binding domain stably expressed in CHO cells. *Biochem. Biophys. Res. Commun*. 384, 486-490. PMC2750803
10. **Du, L.**, Zhao, G., Chan, C., He, Y., Zhou, Y., Zheng, B.J., **Jiang, S.** (2010). A 219-mer recombinant receptor-binding domain of SARS-CoV spike protein induces highly potent immune responses and protection. *Viral. Immunol*. 23, 211–219. PMC2883479

Related patent:

Jiang, S. and He, Y. Neutralizing monoclonal antibodies against severe acute respiratory syndrome-associated coronavirus. *US Patent 7,629,443*. Filed February 7, 2005. Issued December 8, 2009.

D. Research Support

Ongoing Research Support

3R01 AI046221-09S1 Jiang (PI) 6/1/2000 – 5/31/2011
Rational design of HIV fusion inhibitors targeting gp41 (Recovery Act Funds for Administrative Supplements)
The goal of this ARRA project is to determine the binding site of the identified small molecule HIV-1 fusion inhibitors in HIV-1 gp41 coiled coil domain by drug resistance-related mutation studies. **Role:** PI

Completed Research Support

1R01 AI068002-04 Jiang (PI) 3/15/2007 – 2/28/2011
SARS-CoV S protein receptor-binding domain-based vaccines
The goal of this project is to express RBD of SARS-CoV S protein in *E. coli* or yeast systems as subunit vaccines for prevention of SARS. **Role:** PI

2R01 AI046221-09 Jiang (PI) 2/1/2000 – 1/31/2011
Rational design of HIV fusion inhibitors targeting gp41
The goal of this project is to identify lead antiviral compounds targeted to the HIV-1 gp41 coiled coil domain and to optimize the lead compounds for generating most active HIV-1 fusion inhibitors. **Role:** PI

U19 AI076964-05 Jiang (PI) 9/1/04 – 8/31/10 (NCE)
Anti-HIV composite cellulose acetate phthalate film
The major goal of this program project is to evaluate the anti-HIV-1 activity of CAP film in *in vitro* systems, animal models, and phases I clinical trials. **Role:** PI

BIOGRAPHICAL SKETCH

NAME Maria Elena Bottazzi		POSITION TITLE Professor	
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
National University of Honduras, Tegucigalpa, Honduras	BS	1989	Microbiology and Tropical Medicine
University of Florida, Gainesville, FL	Ph.D.	1995	Molecular Biology and Tumor Virology
University of Miami, Miami, FL	Post-doc	1995-1998	Cell Biology, Cell Cycle Genetics
University of Pennsylvania, Philadelphia, PA	Post-doc	1998-2001	Cell Biology and Cell Cycle Genetics

A. Personal Statement

I have extensive experience in the product development activities for recombinant biologics including projects to express proteins derived from hookworm, schistosome and other neglected infectious agents that are vaccine targets. Our studies will involve expression, process development, analytical and formulation development of recombinant expression of the rRBD antigen of SARS-CoV. I have the expertise, leadership and motivation necessary to successfully carry out the proposed work. I have a broad background in molecular and cellular biology, with specific training and expertise in key research areas for this application. I have over 11 years of molecular/cellular biology experience and have served for the last 10 years as the Program Director for Product Development of a recombinant vaccine product development program. I have ample expertise in managing complex programs and technical units for the process development of recombinant vaccines using the yeast and bacteria expression systems. As PI or co-Investigator on several previous non-federal- and NIH-funded grants, I laid the groundwork for the proposed research by developing feasibility processes for recombinant protein expression as well as how to scale them up. In addition, I successfully administered the projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced several peer-reviewed publications from each project. As a result of these previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. The current application builds logically on my prior work and prior product development and manufacturing collaborations.

B. Positions and Honors**Positions and Employment**

1998-1989	Research Assistant Dept of Microbiology, National Univ. of Honduras, Tegucigalpa, Honduras
1989-1995	Graduate Assistant, Dept of Pathology, University of Florida, Gainesville, FL
1995-1998	Post-doctoral Fellow, Dept of Cell biology and Anatomy, Univ. of Miami, Miami, FL
1998-2001	Post-doctoral Fellow, Dept of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA
2001-2004	Assistant Research Professor, Department of Microbiology and Tropical Medicine, The George Washington University, Washington, DC
2004-2007	Associate Research Professor, Department of Microbiology and Tropical Medicine, The George Washington University, Washington, DC
2007-2011	Associate Professor and Department Vice Chair for Administration, Department of Microbiology, Immunology and Tropical Medicine, The George Washington University, Washington, DC
2011-Present	Professor and Associate Dean, National School of Tropical Medicine, Division Pediatric Tropical Medicine, Dept. of Pediatrics, Baylor College of Medicine and Texas children's hospital, Houston, TX

Other Experience and Professional Memberships

2001-	Member, American Society for Microbiology
2001-	Member, American Society for Tropical Medicine and Hygiene
2008-	Associate Editor, Public Library of Science –Neglected Tropical Disease

Honors

BS with Honors, 1989
 American Association of University Women Educational Foundation Pre-Doctoral Fellowship, 1991
 American Heart Association, Florida Affiliate Post-Doctoral Fellowship, 1996

Department of Defense (DOD) Breast Cancer Post-Doctoral Fellowship, 1997

C. Selected Peer-reviewed Publications

Most relevant to the current application

Jariwala AR, Oliveira LM, Diemert DJ, Keegan B, Plieskatt JL, Periago MV, **Bottazzi ME**, Hotez PJ, Bethony JM. Potency testing for the experimental Na-GST-1 hookworm vaccine. *Expert Rev Vaccines*. 2010 Oct; 9(10):1219-30. PMID: 20923271 <http://www.ncbi.nlm.nih.gov/pubmed/20923271>

Zhan B, Perally S, Brophy PM, Xue J, Goud G, Liu S, Deumic V, de Oliveira LM, Bethony J, **Bottazzi ME**, Jiang D, Gillespie P, Xiao SH, Gupta R, Loukas A, Ranjit N, Lustigman S, Oksov Y, Hotez P. (2010). Molecular Cloning, Biochemical Characterization and Partial Protective Immunity of the Heme-binding Glutathione S-Transferases from the Human Hookworm *Necator americanus*. *Infect Immun*. 2010 Feb 9. [Epub ahead of print] PMID: PMC2849424.

Cai S, He F, Samra HS, de la Maza LM, **Bottazzi ME**, Joshi SB, Middaugh CR. (2009). Biophysical and stabilization studies of the *Chlamydia trachomatis* mouse pneumonitis major outer membrane protein. *Mol Pharm*. Sep-Oct;6(5):1553-61. PMID: PMC2757499.

Pearson MS, Bethony JM, Pickering DA, de Oliveira LM, Jariwala A, Santiago H, Miles AP, Zhan B, Jiang D, Ranjit N, Mulvenna J, Tribolet L, Plieskatt J, Smith T, **Bottazzi ME**, Jones K, Keegan B, Hotez PJ, Loukas A. (2009) An enzymatically inactivated hemoglobinase from *Necator americanus* induces neutralizing antibodies against multiple hookworm species and protects dogs against heterologous hookworm infection. *FASEB J*. Sep;23(9):3007-19. PMID: PMC2735369.

Bottazzi, ME and Shah Brown, A. (2008) Model for the product development of vaccines against neglected tropical diseases: a vaccine against human hookworm **Expert Reviews in Vaccines** 7(10):1481-92. PMID: 19053205 <http://www.ncbi.nlm.nih.gov/pubmed/19053205>

Fujiwara RT, Zhan B, Mendez S, Loukas A, Bueno LL, Wang Y, Plieskatt J, Oksov Y, Lustigman S, **Bottazzi ME**, Hotez P, Bethony JM. Reduction of worm fecundity and canine host blood loss mediates protection against hookworm infection elicited by vaccination with recombinant Ac-16. *Clin Vaccine Immunol*. 2007 Mar;14(3):281-7. Epub 2007 Jan 31. PMID: PMC1828853.

Hotez P, Bethony J, **Bottazzi ME**, Brooker S, Diemert D, Loukas A. (2006) New Technologies for the Control of Human Hookworm Infection. *Trends in Parasitology* 22(7):327-31. <http://www.ncbi.nlm.nih.gov/pubmed/16709466>

Bottazzi ME*, Miles AP*, Diemert DJ, Hotez, PJ. (2006) An ounce of prevention on a budget: a non-profit approach to developing vaccines against neglected diseases. *co-first authors. *Expert Reviews of Vaccines* 5(2):189-98. <http://www.ncbi.nlm.nih.gov/pubmed/16608419>

Williamson AL, Lustigman S, Oksov Y, Deumic V, Plieskatt J, Mendez S, Zhan B, **Bottazzi ME**, Hotez P, Loukas, A (2006). Ac-MTP-1, an astascins-like metalloprotease secreted by infective hookworm larvae, is involved in tissue migration. *Infection and Immunity*, 74(2):961-7. PMID: PMC1360348

Fujiwara, R.T., Loukas, A., Mendez, S., Williamson, A.L., Bueno, L.L., Wang, Y., Samuel, A., Zhan, B., **Bottazzi, M.E.**, Hotez, P.J. and Bethony, J.M. (2006). Vaccination with irradiated *Ancylostoma caninum* third stage larvae induces a Th2 protective response in dogs. *Vaccine*, 24(4):501-9. <http://www.ncbi.nlm.nih.gov/pubmed/16140437>

Bottazzi ME*, Goud GN*, Zhan B, Mendez S, Deumic V, Plieskatt J, Liu S, Wang Y, Bueno L, Fujiwara R, Samuel A, Ahn SY, Solanki M, Asojo O, Wen J, Saul A, Bethony JM, Loukas A, Roy M, Hotez PJ (2005) Expression of the *Necator americanus* hookworm larval antigen Na-ASP-2 in *Pichia Pastoris* and purification of the recombinant protein for use in human clinical trials. *co-first authors. *Vaccine*, 23(39):4754-64. <http://www.ncbi.nlm.nih.gov/pubmed/16054275>

Bethony JM, Loukas A, Smout MJ, Mendez S, Wang Y, **Bottazzi ME**, Zhan B, Williamson A, Correa-Oliveira R, Xiao SH, Hotez PJ. (2005) Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *FASEB Journal*, 19(12):1743-5. <http://www.ncbi.nlm.nih.gov/pubmed/16037096>

Loukas A, Bethony JM, Mendez S, Fujiwara RT, Goud GN, Ranjit N, Zhan B, Jones B, **Bottazzi ME**, and Hotez PJ. (2005) Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection. *PLoS Medicine* 2(10):e295. PMID: PMC1240050

D. Research Support

Active

32472 Hotez (PI) 08/01/2000 - 07/31/2011

Human Hookworm Vaccine Initiative 1

Sabin Vaccine Institute is conduit for The Bill and Melinda Gates Foundation

Clinical Development & Evaluation of the Na-ASP-2 Hookworm Vaccine

The goal of this study is to continue product development, including the manufacture of a second pilot lot, and to conduct a global health impact analysis of the human hookworm vaccine with the Sabin Vaccine Institute.

Role: Director Product Development

38988 Hotez (PI) 08/01/2006 - 07/31/2011

Human Hookworm Vaccine Initiative 2

Sabin Vaccine Institute is conduit for The Bill and Melinda Gates Foundation

Development and testing of the Na-APR-1 Hookworm Vaccine

The goal of this study is to conduct the process development, cGMP manufacture and testing, and clinical evaluation of APR-1 in order to develop a bivalent human hookworm vaccine with the Sabin Vaccine Institute

Role: Director Product Development

RCA 8608 Hotez (PI) 09/30/2009 - 09/30/2011 nce

(b)(4) PATH-MVI

Development of a Malaria transmission blocking Vaccine

To develop a feasibility of expression study for the expression and scale-up of the Anopheles-APN-1 target candidate antigen as a malaria transmission blocking vaccine. The goal of this study is to conduct the early feasibility of expression using both yeast and bacterial expression systems.

Role: Director Product Development

1UL1RR031988-01 Hotez (GWU-PI) 07/01/2010 – 06/30/2015

National Institutes of Health

Clinical and Translational Science Institute at Children's National

The goal of the project is to provide highly integrated, cost effective, investigator-focused resources designed to overcome obstacles to investigation, promote collaborative research, and be continuously monitored and evaluated to identify when further adaptation or modification is needed.

Role: Associate Co-Principal Investigator for GWU

1R24TW008897-01 Mullan/Frehywot (PD/PI) 9/01/2010 – 9/29/2015

National Institutes of Health

Coordinating Center: Fostering African Medical Education Community of Excellence

The George Washington University will serve as the Coordinating Center for multiple MEPI programs and linked grantees where the project focuses on improving medical education and research in Sub-Saharan Africa.

Role: Co-Investigator

Dutch Government Bottazzi/Bethony (MPI) 01/01/2011-12/31/2014

Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the Na-GST-1 and Na-APR-1 hookworm antigens in both adults and children.

1R56AI090577-01 Bethony/Bottazzi (MPI) 01/01/2011 – 12/31/2011

National Institutes of Health/Sabin

Product development of a membrane tetraspanin vaccine against schistosomiasis

The goal of this project is to establish a manufacturing process for a vaccine target against schistosomiasis and perform technology transfer for cGMP manufacturing.

Bottazzi/Hotez (MPI) 1/1/2011 - 12/31/2013

Instituto Carlos Slim de la Salud

Slim Initiative for Antipoverty Vaccine Development

The main goal of this project is to build a new generation of urgently needed vaccines for the neglected diseases, and to build capacity for vaccine development in Mexico.

Hotez (PI) 09/01/2011 – 07/31/2016

(b)(4)

Human Hookworm Vaccine Initiative 3

Clinical Development and Evaluation of the Na-GST-1 and Na-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Role: Co-Investigator

Completed

Private Source

Bottazzi (PI)

11/01/2006 - 10/31/2008 on NCE to 10/31/2009

Ancylostoma Caninum derived antigens as potential veterinary diagnostic tools.

The major goal is to explore the possibility of identifying relevant new tools and develop them for the diagnosis of hookworm disease in dogs.

1 R43 CA123991-01A2 Bonafe

06/01/2008 – 05/31/2009

NIH/NCI Phase I SBIR

Glioma therapeutics targeting glioma pathogenesis-related protein

The major goal is to produce recombinant proteins using the yeast *Pichia* eukaryotic system in order to express and purify the glioma pathogenesis-related protein.

R21 AI071903 Kashanchi (PI)

06/01/2006 – 05/31/2007 on NCE to 05/31/2008

NIH/NIAID

A human stem cell mouse model for HIV expression and inhibition

The major goal of this project is to look for human and mouse surface markers from human Stem cells that will be differentiated into human monocyte and T-cells in double KO animals.

Private Source

Greenberg (PI)

11/01/2006 – 03/31/2007 on NCE to 10/31/2007

Private Source

The major goal of this project is a collaborative effort with Private Source to plan for the creation of an International Institute for Public Health Laboratory Management in order to provide training to management, leadership and oversight training to administrators of public health laboratories.

NIH U54 AI57168 Levine (PI)

03/01/2006 – 02/28/2007

MARCE New Opportunities Award

Subcontract to University of Maryland, Baltimore

The major goal of this project is to develop and optimize the expression, purification and scale-up pilot production of HeV and NiV sG recombinant proteins and the human mAbs. The successful process development of these molecules will allow for initiation of preclinical and passive protection studies leading to cGMP manufacture and the clinical testing of cGMP material.

Role: Co-PI

Private Source

Kashanchi (PI)

09/01/2006 – 08/31/2007

The major goal of this project is to identify human immune cells and HIV-1 replication in xenotransplanted Rag2^{-/-} γ_c ^{-/-} mice, and evaluate feasibility of topical anti-HIV microbicide efficacy using NRTI, NNRTI, and entry inhibitors against vaginal challenge. Private Source a 501(c)(3) organization located at Private Source that funds HIV research pertaining to microbicides and AIDS in women,

Role: Co-PI

AI061560 Kashanchi (PI)

06/01/2004 – 05/31/2006

NIH-R21

Title: Alteration of Retinoblastoma tumor suppressor protein in HTLV-1 transformed cells.

The major goal of this project is to understand how tax-dependent perturbation of restriction point regulators leads to leukemogenesis.

Role: Co-PI

Hotez (PI)

08/01/2001-07/31/2003

NIH-NIAID

Hookworm Larval Infectivity and Development.

Role: Research Faculty Supplement for Underrepresented Minorities

DAMD17-97-1-7323 Bottazzi (PI)

10/30/1997 – 07/15/2001

Department of the Army

Anchorage Dependent and Independent control of the cyclin A gene: Implications for breast cancer.

The major goal of this postdoctoral grant was to analyze the regulation of expression of the cyclin A gene in fibroblast as well as mammary epithelial cells leading to the understanding of the progression to breast cancer.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Sara Lustigman	POSITION TITLE Member and Laboratory Head
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
Hebrew University of Jerusalem, Israel	BSc	1975	Chemistry/ biochemistry
Hebrew University of Jerusalem, Israel	MSc	1978	Microbiology/Parsitology
Hebrew University of Jerusalem, Israel	PhD	1985	Immunoparasitology
The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia	Postdoc-Fellow	1988	Malaria Studies

A. Personal Statement

The studies of the proposed research will lead to the expression, process development, formulation, technology transfer, cGMP manufacture, GLP toxicology and preparation of a regulatory filing (IND submission) of a recombinant vaccine to prevent severe acute respiratory distress syndrome (SARS) caused by the SARS-associated coronavirus (SARS-CoV). The vaccine will be comprised of the receptor binding domain (RBD) of the SARS S protein, expressed as a recombinant protein (rRBD), and formulated with alum and/or an innate adjuvant, GLA. The Ultimate Goal of the proposed studies is to develop a highly effective and safe anti-SARS recombinant protein vaccine that can be used in humans. I have a broad background in molecular parasitology, immunology, vaccinology and functional genomics. In the last 20 years my studies, mostly funded by the NIH, were focused on identifying vaccine and drug targets against the filarial human parasitic nematodes. Over the years we identified more than 30 *O. volvulus* antigens of which 8 were proven to be protective in the mouse model, and they are presently being further developed for human use in collaboration with Dr. Peter Hotez and Dr. Bin Zhan (Director of the Molecular Biology Unit of the Human Hookworm Vaccine Initiative) and DR. Maria Elena Bottazzi (Program Director for Product Development), who are both Co-Investigators on my NIH 1R01AI078314-01A2 project. My collaboration with Drs. Peter Hotez, Bin Zhan and Maria Elena Bottazzi has started more than 10 years ago but since 2005 I have been also actively involved in their Hookworm Vaccine Initiative as a collaborator and advisor. Our collaboration resulted in multiple publications and collaborative work on moving forward filarial vaccine candidates through product development and pre-clinical studies. In addition, I am currently also collaborating with Drs. Hotez and Zhan on the product development of a novel parasite derived adjuvant (Ov-ASP-1). To further establish its adjuvanticity we have been using different vaccine models. In collaboration with Dr. Shibo Jiang (PI#2), Head of Viral Immunology we studied since 2005 two vaccine models of SARS, the Spike and the RBD vaccine antigens, as well as the influenza vaccines. Our collaboration is synergistic, complementary and productive because of Dr. Jiang's strong expertise in viral vaccine studies. So far, we have had six jointed publications. My lab has also established varied techniques for studying the adjuvanticity and immunogenicity of vaccine components in terms of antibody responses as well as cellular immune responses elicited against the by-stander vaccine antigens, particularly those against RBD. Most recently, we have been able to show that our adjuvant can support the production of anti-RBD responses in non-human primates. The current application builds logically on my prior work, and the productive collaboration with Drs. Hotez's and Jiang groups. My group will be responsible for testing the immunogenicity of rRBD protein to induce MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte response by using ELISPOT and other alternative assays. In addition, I will be the NYBC liaison for all aspects involving the product development of recombinant RBD and adjuvant formulation for optimization.

B. Positions and Honors

Positions and Employment

3/88-9/94	Assistant Member, Laboratory of Virology and Parasitology, the Lindsley F. Kimball Research Institute (LFKRI), New York Blood Center (NYBC)
10/94-3/98	Associate Member, Laboratory of Virology and Parasitology, LFKRI, NYBC
4/98-12/00	Associate Member and Laboratory Head, Laboratory of Molecular Parasitology, LFKRI, NYBC
1/01-present	Member and Laboratory Head, Laboratory of Molecular Parasitology, LFKRI, NYBC

Other Experience and Professional Memberships

1991 - 1992	Committee Member, Immunodiagnosis of Onchocerca and Filariasis, World Health Organization
1995 - 2000	Scientific Advisory Group Member, Filarial Genome Project, World Health Organization
1998 - 2004	Scientific Advisory Board Member, Beijing Center for Molecular Parasitology, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China
2002	Scientific Advisory Member, Conference on the Eradicability of Onchocerciasis; the Carter Center, Atlanta, GA.
2003	October 15-17, member of a special panel on RNAi in <i>C. elegans</i> as a mean of identifying drug targets for filariasis; World Health Organization, Glion, Switzerland
2003	December 15-17, member of NIAID Special Emphasis Panel (ZAI1-AC-M(J3) & ZAI1-AC-M(J2))
2003 - 2006	ASTMH Scientific Program Committee – Filariasis
2006	February 16, member of NIAID Special Emphasis Panel (ZRG1 IDM-M02 M)
2006, 2007	Member, Scientific Working Group on Helminth Drug Initiative; World Health Organization
2006 -	A member of the Advisory Board of the Worm Institute for Research and Medicine (WIRM), The Scripps Research Institute.
2006 -	Deputy Editor, PLoS NTD (Neglected Tropical Diseases)
2006 -	A member of the Scientific Advisory Board of Filariasis Research Reagent Resource Center (FR3), sponsored by the NIH/NIAID.
2007 -	Editorial Board Member, Molecular and Biochemical Parasitology
2008	October 6-8, member of NIAID Special Emphasis Panel - ZAI1 DDS-M (J2)
9/2009 -	Chairperson of Disease Reference Group on Helminth infections (DRG4), UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR)
2010	January 25-28, Chairperson and member of the NIAID Special Emphasis Panel - ZAI1 AWA-M-M2

C. Selected Peer-reviewed Publications (Selected from 94 peer-reviewed publications)

Most relevant to the current application (in chronological order)

- 1) MacDonald AJ, Cao L, He Y, Zhao Q, **Jiang S** and **LUSTIGMAN S** (2005). rOv-ASP-1, a recombinant secreted protein of the helminth *Onchocerca volvulus*, is a potent adjuvant for inducing antibodies to ovalbumin, HIV-1 polypeptide and SARS-CoV peptide antigens. *Vaccine*, **23**: 3446–3452. PMID: 15837368
- 2) He Y, Li J, Li W, **LUSTIGMAN S**, Farzan M and **Jiang S** (2006). Cross-Neutralization of Human and Palm Civet SARS Coronaviruses by Antibodies Targeting the Receptor-Binding Domain of Spike Protein. *J. Immunology* **176**: 6085–6092. PMID: 16670317
- 3) He Y, Li J, Heck S, **LUSTIGMAN S** and **Jiang S** (2006). Antigenic and Immunogenic Characterization of Recombinant Baculovirus-expressed Severe Acute Respiratory Syndrome Coronavirus Spike Protein: Implication for Vaccine Design. *J. Virology* **80**: 5757–5767. PMID: 16731915; PMCID: PMC1472569
- 4) He Y, Barker SJ, MacDonald AJ, Yu Y, Cao L, Li J, Parhar R, Heck S, Hartmann S, Golenbock DT, **Jiang S**, Libri NA, Semper AE, Rosenberg WM, **LUSTIGMAN S** (2009). Recombinant Ov-ASP-1, a Th1-biased protein adjuvant derived from the helminth *Onchocerca volvulus*, can directly bind and activate antigen-presenting cells. *J Immunol.* **182**:4005-16. PMID: 19299698

- 5) Zhao G, Du L, Xiao W, Sun S, Lin Y, Chen M, Kou Z, He Y, **LUSTIGMAN S, Jiang S**, Zheng BJ, Zhou Y (2010) Induction of protection against divergent H5N1 influenza viruses using a recombinant fusion protein linking influenza M2e to *Onchocerca volvulus* activation associated protein-1 (ASP-1) adjuvant. Vaccine **28**(44):7233-7240. PMID: 20732469

Additional recent publications of importance to the field (in chronological order)

- 1) Abraham D, Leon O, Leon S and **LUSTIGMAN S** (2001). Development of a recombinant antigen vaccine against infection with the filarial worm *Onchocerca volvulus*. Infect. Immun., **69**:262-270. PMID: 16299326; PMID: PMC1307100
- 2) MacDonald AJM, Turaga PSD, Harmon-Brown C, Tierney TJ, Bennett KE, McCarthy MC, Simonek SC, Enyong PA, Moukatté DW and **LUSTIGMAN S** (2002). Differential cytokine and antibody responses to adult and larval stages of *Onchocerca volvulus* consistent with the development of concomitant immunity. Infect. Immun., **70**:2796-2804. PMID: 12010965; PMID: PMC127981
- 3) **LUSTIGMAN S**, MacDonald AJM and Abraham D (2003). CD4⁺ dependent immunity to *Onchocerca volvulus* third-stage larvae in humans and the mouse vaccination model: common ground and distinctions. Int. J. Parasitol., **33**:1161-1171. PMID: 13678632
- 4) Bethony J, Loukas A, Smout M, Brooker S, Mendez S, Plieskatt J, Goud G, **Bottazzi ME**, Zhan B, Wang Y, Williamson A, **LUSTIGMAN S**, Correa-Oliveira R, Xiao S and **Hotez PJ**. (2005). Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. FASEB J, **19**:1743-1745. PMID: 16037096
- 5) Tchakouté VL, Graham SP, Jensen SA, Makepeace BL, Nfon CK, Njongmeta LM, **LUSTIGMAN S**, Enyong PA, Tanya VN, Bianco AE and Trees AJ (2006). In a bovine model of onchocerciasis, protective immunity exists naturally, is absent in drug-cured hosts, and is induced by vaccination. Proceedings of the National Academy of Sciences of the USA **103**: 5971–5976. PMID: 16585501; PMID: PMC1458682
- 6) Fujiwara RT, Zhan B, Mendez S, Loukas A, Bueno LL, Wang Y, Plieskatt J, Oksov Y, **LUSTIGMAN S**, **Bottazzi ME**, **Hotez P** and Bethony JM (2007). Reduction of worm fecundity and canine host blood loss mediates protection against hookworm infection elicited by vaccination with recombinant Ac-16. Clin Vaccine Immunol. **14**:281-287. PMID: 17267592; PMID: PMC1828853
- 7) MacDonald AJ, Libri NA, **LUSTIGMAN S**, Barker SJ, Whelan MA, Semper AE and Rosenberg WM (2008). A novel, helminth-derived immunostimulant enhances human recall responses to hepatitis C virus and tetanus toxoid and is dependent on CD56⁺ cells for its action. Clin Exp Immunol. **152**:265-73. PMID: 18341617; PMID: PMC2384101
- 8) Xiao W, Du L, Liang C, Guan J, **Jiang S, LUSTIGMAN S**, He Y and Zhou Y (2008). Evaluation of recombinant *Onchocerca volvulus* activation associated protein-1 (ASP-1) as a potent Th1-biased adjuvant with a panel of protein or peptide-based antigens and commercial inactivated vaccines. Vaccine, **26**(39):5022-5029. PMID: 18675867; PMID: PMC2597511
- 9) Bergquist R, **LUSTIGMAN S** (2010) Control of important helminthic infections: Vaccine development as part of the solution. Advances in Parasitology, **73**:297-326. PMID: 20627146
- 10) Zhan B, Perally S, Brophy PM, Xue J, Goud G, Liu S, Deumic V, de Oliveira LM, Bethony J, **Bottazzi ME**, Jiang D, Gillespie P, Xiao SH, Gupta R, Loukas A, Ranjit N, **LUSTIGMAN S**, Oksov Y, **Hotez P** (2010). Molecular Cloning, Biochemical Characterization and Partial Protective Immunity of the Heme-binding Glutathione S-Transferases from the Human Hookworm *Necator americanus*. Infection and Immunity, **78**(4):1552-63. PMID: 20145100; PMID: PMC2849424

D. Research Support

Ongoing Research Support

1. 1R01AI078314-01A2 Lustigman (PI) 8/2009 – 7/2014

The development of a recombinant vaccine against human onchocerciasis

The proposal is focused on the preclinical research and development process that will result, through a robust screening process, with the discovery of the best 2 recombinant *O. volvulus* vaccine antigens with the highest

probability for success at inducing protective immunity in humans. The vaccine will target the *O. volvulus* larvae, known to be vulnerable to host immunological attack.

The product development aspect of the project is being done in collaboraiton with the Human Hookworm Vaccine Initiative at the George Washington University and headed by Dr. Peter Hotez. My Co-investigators at the GWU are: Drs. Maria Elena Botazzi and Bin Zhan.

Role:PI

2. R01 AI072465-01A1 Unnasch T (PI) 5/08 – 4/12

Mapping Protein Interactions between Filaria and its Wolbachia Endosymbiont

The overall goal of this proposal will be to identify the proteins that are involved in this endosymbiotic relationship between *Wolbachia* and the filarial parasite *Brugia malayi* and then to establish the functional networks of the genes involved in the process.

Role: Co-Investigator

3. OPP1017584 (Bill & Melinda Gates Foundation) (PI: J. McKerrow) 10/31/2010 – 10/31/2012

Developing a macrofilaricidal drug for onchocerciasis using Anacor's novel oxaborole technology

A collaborative research effort between the University of California San Francisco Sandler Center, Anacor Pharmaceuticals and LFKRI of the NYBC to discover new drug therapies for the treatment of river blindness (onchocerciasis). The collaboration's goal is to identify a novel, potent macro-filaricidal drug candidate that is capable of killing adult worms.

Role: Co-Investigator

4. (b)(4) Pilot Research Project Lustigman (PI) 11/09 – 6/11

Nonhuman primate pilot study of a novel protein adjuvant for vaccines against human pathogens

To maximize the possible therapeutic applications of rOv-ASP-1 as an immunopotentiating adjuvant for human use, we will test its adjuvanticity in a higher animal species and determine whether a reasonable dose can be safely and effectively used in non-human primates (6 animals).

Role: PI

Completed Research Support

1. 1R56AI069547-01A2 Lustigman S (PI) 9/1/2008- 2/28/2010

Malaria in Brazil: RBC variants & parasite invasion

To establish whether molecular variation in the *GYPB* gene, particularly the one that generates the *GYPB**S/s alleles, influences host susceptibility to infection with *P. falciparum* in Brazilian Amazon endemic regions.

2. 3R56AI069547-01A2S1 Lustigman (PI) 9/1/2009 – 2/28/2010

Malaria in Brazil: RBC variants & parasite invasion

To study the association between *P. falciparum* ligand polymorphisms and the RBC invasion profiles used by the Amazonian field isolates and/or the ability of the variant ligand to bind to RBCs with defined phenotypes.

3. WHO/TDR: T24/181/103 A50594 Lustigman (PI) 10/15/07 – 2/28/09

Discovery of novel filaria drug targets using comparative genomics and RNA interference

To identify a high priority targets for macrofilaricide drug discovery using RNAi of target genes identified using bioinformatic filters to be essential for the development of the filarial nematode *Brugia malayi*.

4. R03 TW07349-01 Lustigman (PI) 7/1/2005 – 6/30/2009

Malaria Invasion via Glycophorin B in Brazilian Isolates

The establish the use of GPB mediated pathway of invasion in field parasite isolates from Porto Velho, Rondonia and then validate the importance of distinct GPB variant regions for invasion into RBCs.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Chien-Te Kent Tseng	POSITION TITLE Associate Professor		
eRA COMMONS USER NAME eRA Commons User Name			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
National Chung-Hsing University, Taiwan	B.S.	1974-77	Plant Pathology
Mississippi State University, Starkville, MS	M.S.	1979-81	Immunobiology
University of Arkansas for Medical Sciences, Little Rock	Ph.D.	1994-97	Immunology

A. Personal Statement

The goal of this proposal is to evaluate the efficacy of a subunit of SARS-CoV S protein, termed Receptor Binding Domain (RBD) protein, as a lead candidate vaccine against SARS-CoV infection both in *vitro* and in two well-established mouse models for lethal SARS-CoV infection. Specifically, we plan to establish the best strategy to 1) express the recombinant (r) RBD protein, 2) optimize the vaccination regimens, 3) evaluate the duration of the immunogenicity, 4) protect against diseases and mortality associated with SARS-CoV infection and, finally, 5) minimize or even eliminate the concern of vaccination-induced enhancement of diseases. I have the expertise, leadership, and hands-on experience to successfully carry out the proposed studies with my team members at UTMB in testing the efficacy in mouse models. These two lethal mouse models for SARS-CoV infection are wild-type (*wt*) *Balb/c* mice challenged with mouse-adapted (MA) SARS-CoV (MA-15) and transgenic mice expressing human angiotensin-converting enzyme 2 and challenged with clinical isolates of SARS-CoV, designated “*wt* mice/MA-15” and “ACE2 Tg⁺ mice/SARS-CoV,” respectively. Specifically, we will initially employ the *wt* mice/MA-15 model to evaluate the protective efficacy of the recombinant (r) RBD-based vaccine following an optimized vaccination regimen with regard to antigen dose/s, vaccination frequency and intervals, as well as adjuvant formulation. Once we have achieved a positive outcome in the “*wt* mice/MA-15” model, we will validate, by using the other mouse model (i.e., ACE2 Tg⁺ mice/SARS-CoV), the protective efficacy of the candidate vaccine against both homologous and heterologous clinical isolate/s of SARS-CoV. To assess the efficacy of this rRBD-based vaccine in mice, we will determine 1) titers of RBD-specific, non-neutralizing antibodies, 2) magnitudes of specific T cell responses in vaccinated mice prior to viral challenge, 3) yields of infectious progeny viruses within the lungs, 4) lung pathology emphasizing an assessment of eosinophilic infiltration (if any), 5) clinical manifestations (e.g., weight loss), and, finally, 6) the accumulated mortality-rate in vaccinated versus unvaccinated mice. All of the methodologies required for the proposed studies have been well-established in our laboratories. The UTMB team is comprised of highly experienced scientists (and their staff) from the Departments of Microbiology and Immunology and Pathology, the Center for Biodefense and Emerging Diseases, and the Sealy Center for Vaccine Development (SCVD). This multidisciplinary team has the required experience and complementary expertise to undertake this important task. Furthermore, the team has a well-established, collaborative track-record specifically on the mouse models for SARS since 2003. The Galveston National Laboratory at UTMB, where the proposed work will be conducted, has the necessary infrastructure in terms of state-of-the-art animal and bio-containment facilities required to effectively accomplish the proposed studies within the time-frame proposed. With the support of various funding sources (NIH/NIAID and private sectors), I have been working on vaccine and antiviral evaluation over the past few years and have gained substantial experience. With my experience, dedication, and enthusiasm on SARS research, I feel confident to lead this UTMB SARS research team to complete the experiments proposed for this RO1 application in a timely, thorough, and professional manner.

B. Positions and Honors

1981-1985	Research Assistant	Poultry Disease Research Center, Athens, Georgia
1985-1988	Research Associate	Alton Ochesner Medical Foundation, New Orleans, LA
1988-1993	Senior Research Associate	University of Texas Health Science Center at Houston, TX
1993-1994	Research Scientist	University of Arkansas for Medical Sciences, Little Rock, AR
1994-1997	Ph.D. student	University of Arkansas for Medical Sciences, Little Rock, AR
1997-2001	Postdoctoral Fellow	University of Texas Medical Branch, Galveston, TX
2001-2003	Instructor	University of Texas Medical Branch, Galveston, TX
2003-2007	Assistant Professor	University of Texas Medical Branch, Galveston, TX
2007-Present	Associate Professor	University of Texas Medical Branch, Galveston, TX

C. Selected Peer-reviewed Publications (From a total of 36)

1. Wack, A., E. Soldaini, **C. K. Tseng**, S. Nuti, G.R. Klimpel, and S. Abrignani. 2001. Binding of the hepatitis C envelope protein E2 to CD81 provides a costimulatory signal for human T cells. *Eur. J. Immunol.* 31:166-175. [<http://www.ncbi.nlm.nih.gov/pubmed/11169450>]
2. **Tseng, C.K.**, E. Miskovsky, M. Houghton, and G.R. Klimpel. 2001. Characterization of liver TCR $\gamma\delta$ ⁺ T cells obtained from individuals chronically infected with hepatitis C virus (HCV). Evidence for these T cells playing a role in the liver pathology associated with HCV infections. *Hepatology* 33: 1312-1320. [<http://www.ncbi.nlm.nih.gov/pubmed/1134326>]
3. **Tseng, C.K.**, and G.R. Klimpel. 2002. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer (NK) cell functions. *J. Exp. Med.* 195: 43-50. [PMCID: 2196015]
4. **Tseng, C.K.**, L. Perrone, H. Zhu, S. Makino, and C.J. Peters. 2005. Severe acute respiratory syndrome and the innate immune responses: Modulation of effector cell function without productive infection. *J. Immunol.* 174: 7977-7985. [<http://www.ncbi.nlm.nih.gov/pubmed/15944304>]
5. **Tseng, C.K.**, J. Tseng, L. Perrone, M. Worthy, V. Popov, and C.J. Peters. 2005. Apical entry and release of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) in polarized calu-3 lung epithelial cells. *J. Virology* 79: 9470-9479. [PMCID: 1181546]
6. Yang, S., S.-J. Chen, M.-F. Hsu, J.-D. Wu, **C.K. Tseng**, Y.-F. Liu, H.-C. Chen, C.-W. Kuo, C.-S. Wu, L.-W. Chang, W.-C. Chen, S.-Y. Liao, T.-Y. Chang, H.-H. Hung, H.-L. Shr, C.-Y. Liu, Y.-A. Huang, L.-Y. Chang, J.-H. Hsu, C.J. Peters, A. H.-J. Wang, and M.-C. Hsu. 2006. Synthesis, crystal structure, structure-activity relationships, and antiviral activity of a potent SARS coronavirus 3CL protease inhibitor. *J. Med. Chemistry* 49:4971-80. [<http://www.ncbi.nlm.nih.gov/pubmed/16884309>]
7. Huang, C., N. Ito, **C.K. Tseng**, and S. Makino. 2006. Severe Acute Respiratory Syndrome Coronavirus 7a Accessory Protein Is a Viral Structural Protein. *J. Virology* 80: 7287-7294. [PMCID: 1563709]
8. **Tseng, C.T.**, Huang, C. Newman, P., Wang, N., Narayanan, K., Watts, D.M., Makino, S., Packard, M.M., Zaki, S.R., Chan, T.S. and Peters, C.J. 2007. Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human angiotensin-converting enzyme 2 virus receptor. *J. Virology* 81: 1162-73. [PMCID: 1797529]
9. Santhana G. Devaraj, N. Wang, Z. Chen, Z. Chen, M. Tseng, N. Barretto, R. Lin, C.J. Peters, **C. K. Tseng**, S. Baker, and K. Li 2007. Regulation of IRF-3-dependent Innate Immunity by the Papain-like Protease Domain of the Severe Acute Respiratory Syndrome Coronavirus. *J. Biol. Chem.* 282: 32208-32221. [PMCID: 2756044, NIHMSID129989]
10. Narayanan, K., C. Huang, K.G. Lokugamage, W. Kamitani, T. Ikegami, **C.K. Tseng**, and S. Makino. 2008. Severe Acute Respiratory Syndrome Coronavirus Nsp1 Suppresses Host Gene Expression, Including Type I Interferon, in Infected Cells. *Journal of Virology* 82:4471-9. [PMCID: 2293030]
11. Guangyu Li, Nan Wang, Hilda Guzman, Elena Sbrana, Tomoki Yoshikawa, Chien-Te Kent Tseng, Robert B. Tesh, and Shu-Yuan Xiao. 2008. Dhori Virus (*Orthomyxoviridae: Thogotovirus*) Infection of Mice Produces a Disease and Cytokine Response Pattern Similar That of Highly Virulent Influenza A (H5N1). *American Journal of Tropical Medicine & Hygiene* 78:675-80. [<http://www.ncbi.nlm.nih.gov/pubmed/18385368>]
12. Lokugamage, Kumari, N. Yosikawa, N. Ito, D. Watts, N. Wang, P. Newman, **C.K. Tseng**, C.J. Peters, and S. Makino. 2008. Chimeric coronavirus-like particles carrying severe acute respiratory syndrome coronavirus

(SCoV) S protein protects mice against challenge with SCoV Vaccine 26:797-808. [PMCID: 2267761, NIHMSID39182]

13. Yoshikawa, T., T. Hill, C.J. Peters, and **C.K. Tseng** 2009. Severe acute respiratory syndrome (SARS) coronavirus-induced lung epithelial cytokines exacerbate SARS pathogenesis by modulating intrinsic functions of monocyte-derived macrophages and dendritic cells. *Journal of Virology* 83:3039-48. [PMCID: 2655569]

14. Yoshikawa N, T. Yoshikawa, T. Hill T, C. Huang, D.M. Watts, S. Makino, G. Milligan, T. Chan, C.J. Peters, and **C.K. Tseng**. 2009. Differential virological and immunological outcome of severe acute respiratory syndrome coronavirus infection in susceptible and resistant transgenic mice expressing human angiotensin-converting enzyme 2. *Journal of Virology* 83:5451-65. [PMCID: 2681954]

15. Yoshikawa T, Hill TE, Yoshikawa N, Popov VL, Galindo CL, et al. (2010) Dynamic Innate Immune Responses of Human Bronchial Epithelial Cells to Severe Acute Respiratory Syndrome-Associated Coronavirus Infection. *PLoS ONE* 5(1): e8729. doi:10.1371/journal.pone.0008729 [PMCID: 2806919]

D. Research Support

Ongoing Research Support:

1. Private Source Pls: Womack, J.E. and Peters, C.J.) 07/01/10-06/30/11

Project title: Finding a novel mammalian gene for resistant to Rift Valley fever virus

Role: Co-Investigator EFFORT

2. NIH/NIAID (1R21 AI072201-01A1): NCE- 09/01/10-08/31/11

Project title: Inflammatory Responses and SARS Pathogenesis: An *In Vitro* Model.

Role: PI EFFORT

Completed Research Support:

1. NIH/NIAID (NO1- AI-30039: Couch, Robert)

07/01/05-12/31/10

Subcontract- Viral Respiratory Pathogens Research Unit (VRPRU-SARS)

The purpose is to conduct collaborative research on SARS and SARS coronavirus in an established in vitro model and in small animals for evaluating the protective efficacy of candidate vaccines.

Role: Co-investigator EFFORT

2. NIH/NIAID (NO1- AI25489: Bob Tesh):09/01/09-12/31/10 (Extension with fund)

US Based collaboration in emerging viral and prior diseases

Role: Co-investigator EFFORT of Project 3: Pathogenesis of SARS-CoV and Dhori virus (PI: CJ Peters)

3. NIH/NIAID-WRCE/Career development grant: funding extended to 2/28/10

Project title: Antiviral agents as Therapy for SARS.

Role: P.I. EFFORT

4. NIH/NIAID (NO1- AI25489: Bob Tesh):09/30/02-08/31/10

US Based collaboration in emerging viral and prior diseases

Role: Co-investigator EFFORT of Project 3: Pathogenesis of SARS-CoV and Dhori virus (PI: CJ Peters).

5. Contract/Private Source (10/01/09-9/30/10)

Project title: Genome-wide RNA interference analysis of viral-host interactions"

Role: P.I. EFFORT

6. Contract/Private Source 10/01/09-04/30/10)

Project title: Evaluation of the protective efficacy of neutralizing antibodies raised against hydrophobic heptad repeat (HR) of the Spike protein of coronaviruses against SARS-CoV infection in transgenic mice.

Role: P.I. EFFORT

7. Contract/Private Source 07/01/07-12/31/09

Project title: Micro-neutralization test for the samples obtained from vaccinated animals.

8. Contract/AlphaVax (07/01/08-8/31/09)

Project title: Evaluation of the efficacy of recombinant VEE-based vaccines against SARS- CoV infection in transgenic mice

Role: P.I. EFFORT

9. NIH/NIAID (R21 AI-063118-01: Chan, Tehsheng): 09/01/05-08/31/07

Project title: SARS: A transgenic mouse model for pathogenesis, treatment, and vaccine studies. Role: Co-investigator EFFORT

10. NIH/NIAID (NO1-AI-15435: Chan, Tehsheng): 09/14/06 – 03/31/08

Project title: SARS Lung Mouse Model

Role: Co-investigator [EFFORT]

11. [Private Source] Tseng, Chien-Te Kent): 09/01/05-06/30/07

Project title: Evaluation of anti-SARS compounds in animal models

The contractor will evaluate the efficacy of selected compounds developed by TaiGen Biotechnology against SARS-CoV replication and/or associated pathogenesis in newly established mouse models and hamsters.

Role: PI [EFFORT]

12. [Private Source] (C.J. Peters) 01/06/05-09/29/06

Preparation of inactivated SARS antigen

This is a subcontract for the manufacture of bulk samples of inactivated SARS-CoV strain Urbani.

Role: Co-investigator [EFFORT]

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Du, Lanying		POSITION TITLE Assistant Member	
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
Peking Union Medical College, Beijing, China	B.S.	1995	Basic Medicine
Peking University, Beijing, China	M.S.	2003	Pathogenic Biology
The University of Hong Kong, Hong Kong, China	Ph.D.	2007	Microbiology & Immunology
Lindsley F. Kimball Research Institute			
New York Blood Center, New York	Postdoctoral	2010	Immunology & Virology

A. Personal Statement

The goal of the proposed study is to develop a highly promising lead candidate vaccine against severe acute respiratory syndrome (SARS), the first new human infectious disease of this century classified by NIAID as a Category C biodefense. The vaccine will be designed based on the receptor binding domain (RBD) of SARS-coronavirus (SARS-CoV) spike (S) protein, a region that induced highly potent neutralizing antibodies and protection against virus infection. The vaccine candidate will be further scaled up, characterized, and tested for stability, then goes to technology transfer, cGMP manufacture, GLP toxicology and investigational new drug (IND) preparation and filing. I have strong scientific background in microbiology, molecular biology, cell biology, immunology and other related fields. As the Co-Investigator and an Assistant Member in the laboratory of Dr. Shibo Jiang, the PI#2 of this project, I currently focus my research on the development of recombinant protein vaccines against emerging infectious diseases, particularly SARS and influenza, and evaluated the immune efficacy of these vaccines in different animal models. With extensive experience in protein expression and purification, detection of pseudovirus-based neutralizing activity as well as cross-protection against virus infection, I have developed several important SARS subunit vaccines based on the recombinant RBD protein of SARS-CoV in different expression systems and demonstrated their high potency in inducing strong neutralizing antibodies and complete protection against virus challenge. My research findings related to SARS have been published in around 20 peer-reviewed journals and presented in a number of national and international conferences. My strong background and extensive experience in SARS study have demonstrated my ability to serve as the key researcher in the proposed project.

B. Positions and Honors**Positions and Employment**

- 1995-96 Research Assistant, Beijing BioTinge-Tech Center for Biotechnology, Chinese Academy of Medical Sciences, Beijing, China
- 1996-99 Senior Researcher, Dept. of Biochemistry and Clinical Diagnosis, Beijing Company of Modern Bio-Technology LTD, Beijing, China
- 1999-03 Pre-doctoral researcher, School of Basic Medical Sciences, Peking University Health Science Center, Peking University, Beijing, China
- 2002-03 Research Assistant, Vascular Bioengineering Laboratory, Dept. of Surgery and Bioengineering, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

- 2003-07 Postgraduate, Dept. of Microbiology, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong
- 2007-10 Postdoctoral Research Fellow, Viral Immunology Laboratory, Lindsley F. Kimball Research Institute, New York Blood Center, NY, USA
- 2010-11 Senior Research Fellow, Viral Immunology Laboratory, Lindsley F. Kimball Research Institute, New York Blood Center, NY, USA
- 2011- Assistant Member, Viral Immunology Laboratory, Lindsley F. Kimball Research Institute, New York Blood Center, NY, USA

Other Experience and Professional Memberships

- 2001- The Chinese Society of Medical Sciences, Member
- 2001- The Chinese Microbiology Society, Member
- 2005- American Society for Virology, Associate Member
- 2008- American Society for Microbiology, Postdoctoral Member
- 2008- Canadian Journal of Microbiology, Invited Reviewer
- 2008- Viral Immunology, Invited Reviewer
- 2010- American Association of Immunologists, Regular Member
- 2010- New York Academy of Sciences, Member

Honors

- 2003-07 Postgraduate Scholarship Award, the University of Hong Kong
- 2006 Conference Grant Award, the University of Hong Kong, the Committee on Research and Conference Grants (CRCG) for Research Students
- 2006 Travel Grant Award, the 16th European Congress of Clinical Microbiology and Infectious Diseases (16th ECCMID)
- 2006 Student Travel Grant Award, the 2006 American Society for Virology 25th Annual Meeting (25th ASV)
- 2008 Postdoctoral Fellow Travel Grant Award, the 2008 American Society for Virology 27th Annual Meeting (27th ASV)
- 2009 Financial Aid for attending 2009 Cold Spring Harbor Laboratory meeting on harnessing immunity to prevent and treat disease.
- 2010 New York Blood Center 2010 Quarterly Employee Recognition Award

C. Selected Peer-reviewed Publications (Selected from 35 peer-reviewed publications)

Most relevant to the current application (in chronological order)

1. **Du L**, Zhao G, He Y, Guo Y, Zheng BJ, **Jiang S**, Zhou Y. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. *Vaccine*. 2007;25(15):2832-2838. <http://www.ncbi.nlm.nih.gov/pubmed/17092615>
2. **Du L**, Zhao G, Lin Y, Sui H, Chan C, Ma S, He Y, **Jiang S**, Wu CY, Yuen KY, Jin DY, Zhou Y, Zheng BJ. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. *J Immunol*. 2008;180(2):948-956. PMC2603051
3. **Du L**, He Y, Zhou Y, Liu S, Zheng BJ, **Jiang S**. The spike protein of SARS-CoV – a target for vaccine and therapeutic development. *Nature Rev Microbiol*. 2009;7(3):226-236. PMC2750777

4. **Du L**, Zhao G, Li L, He Y, Zhou Y, Zheng BJ, **Jiang S**. Antigenicity and immunogenicity of SARS-CoV S protein receptor-binding domain stably expressed in CHO cells. *Biochem Biophys Res Commun*. 2009;384(4):486-490. PMC2750803
5. **Du L**, Zhao G, Chan CC, Sun S, Chen M, Liu Z, Guo H, He Y, Zhou Y, Zheng BJ, **Jiang S**. Recombinant receptor-binding domain of SARS-CoV spike protein expressed in mammalian, insect and E. coli cells elicits potent neutralizing antibody and protective immunity. *Virology*. 2009;393(1):144-150. PMC2753736

Additional recent publications of importance to the field (in chronological order)

1. **Du L**, He Y, Wang Y, Zhang H, Ma S, Wong CK, Wu SH, Ng F, Huang JD, Yuen KY, **Jiang S**, Zhou Y, Zheng BJ. Recombinant adeno-associated virus expressing the receptor-binding domain of severe acute respiratory syndrome coronavirus S protein elicits neutralizing antibodies: implication for developing SARS vaccines. *Virology*. 2006;353(1):6-16. <http://www.ncbi.nlm.nih.gov/pubmed/16793110>
2. **Du L**, Kao RY, Zhou Y, He Y, Zhao G, Wong C, **Jiang S**, Yuen KY, Jin DY, Zheng BJ. Cleavage of spike protein of SARS coronavirus by protease Factor Xa is associated with viral infectivity. *Biochem Biophys Res Commun*. 2007;359(1):174-179. PMC2323977
3. **Du L**, Zhao G, Lin Y, Chan C, He Y, **Jiang S**, Wu C, Jin DY, Yuen KY, Zhou Y, Zheng BJ. Priming with rAAV encoding RBD of SARS-CoV S protein and boosting with RBD-specific peptides for T cell epitopes elevated humoral and cellular immune responses against SARS-CoV infection. *Vaccine*. 2008;26(13):1644-1651. PMC2600875
4. Xiao W*, **Du L***, Liang C, Guan J, **Jiang S**, **Lustigman S**, He Y, Zhou Y. Evaluation of recombinant onchocerca volvulus activation associated protein-1 (Ov-ASP-1) as a potent Th1-biased adjuvant with a panel of protein or peptide-based antigens and commercial inactivated vaccines. *Vaccine*. 2008;26(39):5022-5029. (*Equal first-authors). PMC2597511
5. **Du L**, He Y, **Jiang S**, Zheng BJ. Development of subunit vaccines against severe acute respiratory syndrome. *Drugs Today (Barc)*. 2008;44(1):63-73. <http://www.ncbi.nlm.nih.gov/pubmed/18301805>
6. **Du L**, Zhao G, Chan C, He Y, Zhou Y, Zheng BJ, **Jiang S**. A 219-mer recombinant receptor-binding domain of SARS-CoV spike protein induces highly potent immune responses and protection. *Viral Immunol*. 2010;23(2):211-219. PMC2883479
7. **Du L**, Zhou Y, **Jiang S**. Research and development of universal influenza vaccines. *Microbes Infect*. 2010;12(4):280-286. <http://www.ncbi.nlm.nih.gov/pubmed/20079871>
8. **Du L**, Zhao G, Zhang X, Liu Z, Yu H, Zheng BJ, Zhou Y, **Jiang S**. Development of a safe and convenient neutralization assay for rapid screening of influenza HA-specific neutralizing monoclonal antibodies. *Biochem Biophys Res Commun*. 2010;397(3):580-585. <http://www.ncbi.nlm.nih.gov/pubmed/20617558>
9. Zhao G, **Du L**, Xiao W, Sun S, Lin Y, Chen M, Kou Z, He Y, **Lustigman S**, **Jiang S**, Zheng BJ, Zhou Y. Induction of protection against divergent H5N1 influenza viruses using a recombinant fusion protein linking influenza M2e to Onchocerca volvulus activation associated protein-1 (ASP-1) adjuvant. *Vaccine* 2010;28(44):7233-7240. <http://www.ncbi.nlm.nih.gov/pubmed/20732469>
10. **Du L**, Leung VH, Zhang X, Zhou J, Chen M, He W, Zhang HY, Chan CC, Poon, VK, Zhao G, Sun S, Cai Li, Zhou Y, Zheng BJ, **Jiang S**. A recombinant vaccine of H5N1 HA1 fused with foldon and human IgG Fc induced complete cross-clade protection against divergent H5N1 viruses. *PLoS One* 2011;6(1):e16555. PMC3029370

Relative patent to the current application

1. **Jiang S, Du L.** Immunopotentiator-linked oligomeric influenza immunogenic compositions. Filed October 05, 2010. *US Serial No.* 61/106,101.
2. **Jiang S, Du L,** Zhou Y. Influenza hemagglutinin-specific monoclonal antibodies for preventing and treating influenza virus infection. Filed October 20, 2010. *US Serial No.* 61/405,100.

D. Research Support

Ongoing Research Support

1R03 AI088449-01A1 Du (PI)

2011 – 2012 NIH/NIAID

Rational design of M2e-FP conserved epitope-based universal influenza A vaccines

The goal of this project is to develop recombinant protein-based universal influenza vaccines using conserved sequences of M2e and HA fusion peptide of H5N1 virus.

Role: PI

Overlap: None

Completed Research Support

1R01 AI68002-04 Jiang (PI)

2007 – 2011 NIH/NIAID

SARS-CoV S protein receptor-binding domain-based vaccines

The goal of this project is to express RBD of SARS-CoV S protein in *E. coli* or yeast systems as subunit vaccines for prevention of SARS.

Role: Research Fellow

Overlap: None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Bin Zhan	POSITION TITLE		
eRA COMMONS USER NAME	Research Professor		
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing,</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Fujian Medical College, Fuzhou, China	M.D.	1983	Medicine
Chinese Academy of Preventive Medicine, Shanghai, China	M.S.	1989	Parasitology
University of Texas Medical Branch at Galveston, Galveston, Texas	Postdoctoral	1994	Molecularbiology

A. Personal Statement

I decided to dedicate myself to the infectious diseases and parasitological research when I realized so many people in my country and other developing countries suffer from the infectious and parasitic diseases during my study in medical college. Soon after I received my MD degree I joined the fight against infectious diseases and parasites with multidisciplinary studies of epidemiology, immunology, and molecular biology of malaria, leishmania and hookworm. Particularly, I have focused my research on developing human hookworm vaccines since 2000 when the Human Hookworm Vaccine Initiative (HHVI) was funded by Private Source

Private Source My research includes the discovery and cloning of novel hookworm antigens by immunological and molecular biological methods, characterizing their biological functions in the survival of hookworm in the host as well as evaluating their potential as protective antigens and/or hookworm vaccines. From my work, more than 40 new hookworm antigens as well as their DNA have been identified and cloned from different species and stages of hookworm. Many of them have been characterized and tested for their vaccine potential. Some of these vaccine candidates are in process development and pilot manufacture for clinic trials. Except for vaccine development for hookworm I also devote myself into the vaccine research against other parasitic infection such as malaria, leishmania, chagas disease and Onchocerciasis. I have more than 60 publications in peer-reviewed journals. Due to my successful and productive research work in molecular biology, immunology and infectious diseases, I am confident that I can co-lead the proposed project with my expertise and experience.

B. Positions and Honors.**Position and Employment**

1983 - 1986	Assistant lecturer, Fujian Medical College, Fuzhou, China
1989 - 1984	Assistant professor, Chinese Academy of Preventive Medicine, Institute of Parasitic Diseases, Shanghai, China.
1994 - 1994	Research Associate, New York Medical College, Valhalla, NY
1994 - 1996	Post Doctoral Fellow, University of Texas Medical Branch, Galveston, TX
1996 - 1997	Visiting scientist, Yale University Medical School, New Haven, CT.
1997 - 2000	Associate Professor, Chinese Academy of Preventive Medicine, Institute of Parasitic Diseases, Shanghai, China.
2000 - 2003	Assistant Research Professor, The George Washington University Medical Center, DC
2004 - 2008	Associate Research Professor, The George Washington University Medical Center, DC
2009 - present	Research Professor , The George Washington University Medical Center, DC

Other Experience and Professional Memberships

- 1998 – 2000 Acting Chief, Department of Pharmacology, Chinese Academy of Preventive Medicine, Institute of Parasitic Diseases, Shanghai, China
- 2001 - Members, American Society of Parasitologist, American Society of Tropical Medicine and Hygiene Sigma XI, The Scientific Research Society

C. Selected peer-reviewed publications (in chronological order).

1. Bin Zhan, Mahnaz Badamchian, Bo Meihua, James Ashcom, Jianjun Feng, John Hawdon, Xiao Shuhua, and Peter J. Hotez. Molecular cloning and purification of Ac-TMP, a developmentally regulated putative tissue inhibitor of metalloprotease released in relative abundance by adult *Ancylostoma* hookworms. *Am. J. Trop. Med. Hyg.* 2002; 66(3):238-244.
2. Bin Zhan, Peter J Hotez, Yan Wang and John M Hawdon. A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of protease. *Mol. Biochem. Parasitol.* 2002; 120:291-296.
3. Sridhar Basavaraju, Bin Zhan, Malcolm W. Kennedy, Yueyuan Liu, John Hawdon and Peter J. Hotez . Ac-FAR-1, a 20 kDa Fatty Acid- and Retinol-Binding Protein Secreted by Adult *Ancylostoma caninum* Hookworms: gene transcription pattern, ligand binding properties and structural characterisation. *Molecular and Biochemical Parasitology.* 2003; 126:63-71.
4. Bin Zhan, Yueyuan Liu, Mahnaz Badamchian, Angela Williamson, Jianjun Feng, Alex Loukas, John M. Hawdon and Peter J. Hotez. Molecular characterization of the *Ancylostoma*-secreted Protein (ASP) family from the adult stage of *Ancylostoma caninum*. *Int. J. Parasitol.* 2003; 33:897-907.
5. Gaddam Narsa Goud, Bin Zhan, Kashinath Ghosh, Alex Loukas, John Hawdon, Azra Dobardzic, Vehid Deumic, Sen Liu, Reshad Dobardzic, Bernard C. Zook, Qun Jin, Yueyuan Liu, Laura Hoffman, Sophia Chung-Debose, Rachna Patel, Susana Mendez, and Peter J. Hotez. Cloning, yeast expression, isolation, and vaccine testing of recombinant *Ancylostoma*-secreted protein (ASP)-1 and ASP-2 from *Ancylostoma ceylanicum*. *J Infect Dis.* 2004; 189(5):919-29.
6. Bin Zhan, Yan Wang, Yueyuan Liu, Angela Williamson, Alex Loukas, John M. Hawdon, Xue Hae-chou, Xiao Shu-hua, and Peter J. Hotez. Ac-SAA-1, an immunodominant 16 kDa surface-associated antigen of infective larvae and adults of *Ancylostoma caninum*. *Int. J Parasitol.* 2004; 34:1037-45.
7. Bin Zhan, Sen Liu, Samirah Perally, Jian Xue, Ricardo Fujiwara, Peter Brophy, Shuhua Xiao, Yueyuan Liu, Jianjun Feng, Angela Williamson, Yan Wang, Lilian L. Bueno, Susana Mendez, Gaddam Goud, Jeffrey M. Bethony, John M. Hawdon, Alex Loukas, Karen Jones and Peter J. Hotez. Biochemical characterization and vaccine potential of a heme binding glutathione transferase (GST) from the adult hookworm *Ancylostoma caninum*. *Infection and Immunity* 2005; 73(10):6903-11.
8. Susana Mendez, Bin Zhan, Gaddam Goud, Kashinath Ghosh, Azra Dobardzic, Wenhui Wu, Sen Liu, Vehid Deumic, Reshad Dobardzic, Yueyuan Liu, Jeff Bethony and Peter J. Hotez. Effect of combining the larval antigens *Ancylostoma* Secreted Protein 2 (ASP-2) and metalloprotease 1 (MTP-1) in protecting hamsters against hookworm infection and disease caused by *Ancylostoma ceylanicum*. *Vaccine.* 2005; 23:3123-30.
9. Jianjun Feng, Bin Zhan, Yueyuan Liu, Sen Liu, Angela Williamson, Gaddam Goud, Alex Loukas, Peter Hotez. Molecular cloning and characterization of Ac-MTP-2, an astacin-like metalloprotease released by adult *Ancylostoma caninum*. *Mol. and Biochem. Parasitol.* 2007; 152(2):132-8.
10. Ricardo Fujiwara, Bin Zhan, Susana Mendez, Alex Loukas, Lilian L. Bueno, Yan Wang, Jordan Plieskatt, Yelena Oksov, Sara Lustigman, Maria Elena Bottazzi, Peter Hotez and Jeffrey M. Bethony. Reduction of worm fecundity and canine host blood loss mediates protection against hookworm infection elicited by vaccination with recombinant Ac-16. *Clin Vaccine Immunol.* 2007;14(3):281-7.
11. Shuhua Xiao, Bin Zhan, Jian Xue, Gaddam Narsa Goud, Alex Loukas Yueyuan Liu, Angela Williamson, Sen Liu, Vehid Deumic, Peter Hotez. The evaluation of recombinant hookworm antigens as vaccines in hamsters (*Mesocricetus auratus*) challenged with human hookworm, *Necator americanus*. *Experimental Parasitology*, 2008;118(1):32-40.
12. Ranjit N, Zhan B, Stenzel DJ, Mulvenna J, Fujiwara R, Hotez PJ, Loukas A. A family of cathepsin B cysteine proteases expressed in the gut of the human hookworm, *Necator americanus*. *Mol Biochem Parasitol.* 2008;160(2):90-9.
13. Zhan B, Gupta R, Wong SP, Bier S, Jiang D, Goud G, Hotez P. Molecular cloning and characterization of

Ac-TMP-2, a tissue inhibitor of metalloproteinase secreted by adult *Ancylostoma caninum*. Mol Biochem Parasitol. 2008 Dec;162(2):142-8

14. Ranjit N, Zhan B, Hamilton B, Stenzel D, Lowther J, Pearson M, Gorman J, Hotez P, Loukas A. Proteolytic Degradation of Hemoglobin in the Intestine of the Human Hookworm *Necator americanus*. J Infect Dis. 2009;199(6):904-12.
15. Bin Zhan, Samirah Perally, Peter M Brophy, Jian Xue, Gaddam Goud, Sen Liu, Vehid Deumic, Luciana M de Oliveira, Jeffrey Bethony, Desheng Jiang, Portia Gillespie, Shu-hua Xiao, Richi Gupta, Alex Loukas, Najju Ranjit, Sara Lustigman, Yelena Oksov, Peter Hotez. Molecular Cloning, Biochemical Characterization, and Vaccine Potential of the Heme-binding Glutathione Transferases from the Human Hookworm *Necator americanus*. Infection and Immunity, 2010, 78(4):1552-63

C. Research Support

Ongoing Research Support

32472 Hotez (PI) 08/01/2000 – 07/31/2011

Human Hookworm Vaccine Initiative 1

The Bill and Melinda Gates Foundation via Albert B. Sabin Vaccine Institute

Human Hookworm Vaccine Initiative (HHVI): Clinical Development & Evaluation of the Na-ASP-2 Hookworm Vaccine

The goal of this study is to continue product development, including the manufacture of a second pilot lot, and to conduct a global health impact analysis of the human hookworm vaccine with the Sabin Vaccine institute.

Role: Unit Director - Hookworm Antigen Discovery

38988 Hotez (PI) 08/01/2006 – 07/31/2011

Human Hookworm Vaccine Initiative 2

Human Hookworm Vaccine Initiative (HHVI): To develop and test the Na-APR-1 Hookworm Vaccine

The goal of this study is to conduct the process development, cGMP manufacture and testing, and clinical evaluation of APR-1 in order to develop a bivalent human hookworm vaccine with the Sabin Vaccine Institute

Role: Unit Director - Hookworm Antigen Discovery

1 R01A1078314-01 Lustigman (PI) 08/25/2009 – 7/31/2014

NIH/NIAID

The development of a recombinant vaccine against human onchocerciasis

The major goal of this subcontract is to clone, express, characterize and optimize the expression of the eight selected *Onchocerca* vaccine candidate antigens (rOvAgs) using the yeast *Pichia* eukaryotic system.

Role: Co-PI

2R01AI056189 - 06A1 Aroian (PI) 08/01/2010 – 07/31/2012

NIH

B. thuringiensis Crystal Proteins as Powerful Next-Generation Anthelmintics

The major goal of this subcontract is to test the effects of different formulated Cry5B against hookworm using *Ancylostoma ceylanicum*/hamster model

Role: Co-PI

Bottazzi/Bethony (MPI) 01/01/2011-12/31/2014

Sponsor Ministry of Foreign Affairs, The Netherlands

Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the Na-GST-1 and Na-APR-1 hookworm antigens in both adults and children.

Role: Director of Molecular Biology

Hotez (PI) 09/01/2011 – 07/31/2016

Sponsor: Private Source

Human Hookworm Vaccine Initiative 3

Title: Clinical Development and Evaluation of the Na-GST-1 and Na-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Role: Director of Molecular Biology

1 R01A1078314-01A2 Lustigman (PI) 08/25/2009 – 07/31/2014

NIH/NIAID (Subcontract from New York Blood Center)

The development of a recombinant vaccine against human onchocerciasis

The major goal of this subcontract is clone, express, characterize and optimize the expression of the eight selected *Onchocerca* vaccine candidate antigens (rOvAgs) using the yeast *Pichia* eukaryotic system.

Role: Co-PI

Completed Research Support

1 R43 CA123991-01A2 Bonafe 06/01/2008 – 05/31/2009

NIH/NCI Phase I SBIR

Glioma therapeutics targeting glioma pathogenesis-related protein

The major goal is to produce recombinant proteins using the yeast *Pichia* eukaryotic system in order to express and purify the glioma pathogenesis-related protein.

Chinese National Natural Science Foundation Zhan (PI) 1992 - 1994

Application of Polymerase Chain Reaction on the diagnosis of malaria. A novel PCR method was developed to diagnose malaria (*Plasmodium falciparum*) with high sensitivity and specificity

P50 AI039461 Feng (PI) 1997 - 2000

NIH/NIAID TMRC in China

Emerging Infectious Diseases in China.

My study part is to perform a epidemiological investigation of hookworm infection in China and identify the genetic variation of hookworm from different parts of China.

Role: Co-PI

Private Source Bottazzi (PI) 11/01/2006 - 10/31/2008 on NCE to 10/31/2009

Ancylostoma Caninum derived antigens as potential veterinary diagnostic tools.

The major goal is to explore the possibility of identifying relevant new tools and develop them for the diagnosis of hookworm disease in dogs.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Teh-sheng Chan		POSITION TITLE Professor	
eRA COMMONS USER NAME eRA Commons User Name			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Natl. Taiwan Univ., College of Medicine	M.D.	1963	Medicine and Surgery
Yale University	Ph.D.	1969	Molecular Biophysics
Rockefeller University	Postdoc	1969-71	Microbial Genetics
Massachusetts Institute of Technology	Postdoc	1971-73	Somatic Cell Genetics

A. Personal Statement

As a co-investigator of this grant application, I will be responsible for breeding, genotyping, and characterizing transgenic mice expressing the human ACE2 gene. Publications 12 and 13 listed below describe the properties of two of the transgenic lineages, and their use as lethal and nonlethal models for SARS infection. Either or both lineages will be suitable for vaccine studies, with various end-points for assessing vaccine efficacies. I am the originator of these lines and have been collaborating with Dr. C.-T. K. Tseng and his group on different SARS research projects since the outbreak of SARS in 2002. The most recent research interest has focused on the role of cytokines in the pathogenesis of SARS by using several lines of mouse knock-out mutants. Thus, our group and that of Dr. Tseng have formed a productive team tackling several aspects of SARS infection.

My other ongoing research project is to develop a new, improved mouse model for SARS, by using knock-in technology.

B. Positions and Honors**Positions and Employment**

1962-1963 Intern, National Taiwan University Hospital, Taipei
 1969-1971 Postdoctoral Fellow, Rockefeller University (Dr. Norton Zinder), New York
 1971-1973 Research Associate, Massachusetts Institute of Technology (Dr. Howard Green), Cambridge
 1973-1978 Assistant Professor, Dept. of Physiology, University of Connecticut Health Center, Farmington
 1978-1993 Associate Professor, Dept. of Microbiology, University of Texas Medical Branch, Galveston
 1993-Present Professor, Dept. of Microbiology and Immunology, University of Texas Medical Branch

Awards and Honors:

1963 *Summa cum laude*, Natl. Taiwan Univ. College of Medicine
 1963 Formosan Medical Association Award
 1969-1972 Helen Hay Whitney Fellowship Award
 1992 The Best Original Research Article Award. *J. Genet. Mol. Biol.*
 1993 The Distinguished Teaching Award, University of Texas Medical Branch

C. Selected Peer-Reviewed Publications (selected from 57 peer-reviewed publications)

1. **Chan, T.-s.** and A. Garen. Amino acid substitution resulting from suppression of nonsense mutations, V. Tryptophan insertion by Su9+ suppressor gene. *J. Mol. Biol.* 49:231-234; 1970. [<http://www.ncbi.nlm.nih.gov/pubmed/4317908>]
2. **Chan, T.-s.**, R. W. Webster, and N. D Zinder. Suppression of UGA codon by a tryptophan tRNA. *J. Mol. Biol.* 56:101-116; 1971. [<http://www.ncbi.nlm.nih.gov/pubmed/4929882>]

3. Green, H. and **T.-s. Chan**. Pyrimidine starvation induced by adenosine in fibroblasts and cells of lymphoid origin: the role of adenosine deaminase. *Science* 182:836-837; 1973. [<http://www.ncbi.nlm.nih.gov/pubmed/4795749>]
4. **Chan, T.-s.** Deoxyguanosine toxicity on lymphoid cells as a cause for immunosuppression in purine nucleoside phosphorylase deficiency. *Cell* 14:523-530; 1978. <http://www.ncbi.nlm.nih.gov/pubmed/99242>
5. **Chan, T.-s.** Purine excretion by macrophages lacking adenosine deaminase activity. *Proc. Natl. Acad. Sci. USA* 76:925-929; 1979. [<http://www.ncbi.nlm.nih.gov/pubmed/311477>]
6. Sun, J., F. Bodola, X. Fan, H. Irshad, L. Soong, S. M. Lemon and **T.-s. Chan**. Hepatitis C virus core and envelope proteins do not suppress the host ability to clear a hepatic viral infection. *J. Virol.*, 25:11992-11998; 2001. [<http://www.ncbi.nlm.nih.gov/pubmed/11711589>]
7. Young, H. W., J. G. Molina, D. Dimina, H. Zhong, M. Jacobson, L. N. Chan, **T.-s. Chan**, J. J. Lee, M. R. Blackburn. A3 adenosine receptor signaling contributes to airway inflammation and mucus production in adenosine deaminase-deficient mice. *J. Immunol.* 173: 1380-1389; 2004. [<http://www.ncbi.nlm.nih.gov/pubmed/15240734>]
8. Sun, J, B. Tumurbaatar, J. Jia, H. Diao, F. Bodola, S. M. Lemon, W. Tang, D. G. Bowen, G. W. McCaughan, P. Bertolino and **T.-s. Chan**. Parenchymal expression of CD86/B7.2 contributes to hepatitis C virus-related liver injury. *J. Virol.* 79:10730-10739, 2005. [<http://www.ncbi.nlm.nih.gov/pubmed/16057865>]
9. Korenaga, M., **T.-s. Chan**, J. Sun, S. Weinman. Hepatitis C virus core protein inhibits mitochondrial electron transport and increases ROS production. *J. Biol. Chem.* 280:37481-37488, 2005 [<http://www.ncbi.nlm.nih.gov/pubmed/16150732>]
10. Tumurbataar, B., Y. Sun, **T. Chan**, J. Sun. Cre-estrogen receptor-mediated hepatitis C virus structural protein expression in mice. *J. Virol. Methods.* 146: 5-13, 2007. [PMCID: 2104783]
11. Tseng, K. C.-T., C. Huang, P. Newman, N. Wang, K. Narayanan, D. Watts, S. Makino, M. Packard, S. R. Zaki, **T.-s. Chan**, C. J. Peters. Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human angiotensin-converting enzyme 2 virus receptor. *J. Virol.* 81: 1162-1173, 2007. [PMCID: 1797529]
12. Yoshikawa, N., T. Yoshikawa, T. Hill, C. Huang, D. M. Watts, S. Makino, **T.-s. Chan**, C. J. Peters, and C.-T. K. Tseng. Differential virological and immunological outcome of severe acute respiratory syndrome-coronavirus (SARS-CoV) infection of susceptible and resistant transgenic mice expressing human angiotensin-converting enzyme 2. *J. Virol.* 83:5451-5465, 2009. [PMC2681954]
13. Desai, M. M., B. Tumurbataar, Y. Zhang, L.-N. L. Chan, J. Sun, **T.-s. Chan**. Aberrant Transcription and post-transcriptional processing of hepatitis C virus non-structural genes in transgenic mice. *Transgenic Res.* Online first™ 24 February 2011. [<http://www.ncbi.nlm.nih.gov/pubmed/21347690>]
14. Desai, M. M., B. Gong, **T.-s. Chan**, R. A. Davey, L. Soong., A. A. Kolokoltsov, J. Sun. Differential Type I Interferon-Mediated Autophagic Trafficking of Hepatitis C Virus Proteins in Mouse Liver. *Gastroenterology*. In press.

C. Research Support for Past Three Years

Active:

1RO1 AI069142 (PI: J. Sun, Co-I: T. Chan) 12/1/2006-11/31/2011
 Immune mechanism of HCV persistence and pathogenesis
 The aim is to delineate the immunopathogenesis of hepatitis C using transgenic mouse models

Completed:

1 R21AI063118-01A1 (PI: Chan) 07/01/2005-06/30/2008
 NIH/NIAID
 Transgenic Mouse Models for Pathogenesis and Therapy
 We propose to develop transgenic mouse lines to develop a SARS –murine model.

A transgenic mouse strain deficient in adenosine deaminase, which develops asthma-like disease, has been derived. The goal of this grant is to elucidate the mechanism of the asthma-like disease in this mouse model.

014029001 – NIH (PI: T. Chan)

09/14/2006 – 03/31/2008

Subaward from Utah State University (N01AI15435).

SARS lung mouse model

This contract is to develop transgenic mice expressing human ACE2 specifically in the lung for use in SARS research.

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2012 * End Date: 04/30/2013

Budget Period 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.		Peter	J	Hotez	M.D.	PD/PI	Institutional Base Salary	EFFORT			19,970.00	4,593.00	24,563.00
2.		Maria	Elena	Bottazzi	PhD	PD/PI					39,940.00	9,186.00	49,126.00
3.		Bin		Zhan	MD	Co-I					12,531.00	2,882.00	15,413.00
4.													
5.													
6.													
7.													
8.													
9. Total Funds requested for all Senior Key Persons in the attached file													
Total Senior/Key Person													89,102.00

Additional Senior Key Persons:

Add Attachment

Delete Attachment

View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Scientist for Molecular Biology	EFFORT			58,066.00	17,420.00	75,486.00
1	Associate Director for Product Development				30,750.00	9,225.00	39,975.00
1	Director, Process Development				4,870.00	1,461.00	6,331.00
1	Research Assistant				29,904.00	8,971.00	38,875.00
4	Total Number Other Personnel	Total Other Personnel					160,667.00
Total Salary, Wages and Fringe Benefits (A+B)							249,769.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2012 * End Date: 04/30/2013 Budget Period 1

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

	Equipment item	* Funds Requested (\$)
1.	Floor model refrigerated incubated shaker	9,600.00
2.	Sample application pump, Air Sensor and Buffer Selection kit fo	13,025.00
3.	Circular Dichroism Chiral Dector w/software	31,158.00
4.	Mobius with DLS (Zeta potential instrument with dynamic light s	62,475.00
5.	Acquitivy Fluorescence Detector	14,832.00
6.	Parallel Bioreactor System	140,000.00
7.	Biochemistry Analyzers	13,818.00
8.	Spectrophotometer	8,513.00
9.	Akta Explorer High Flow Kit	5,082.00
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	298,503.00

Additional Equipment:

Add Attachment

Delete Attachment

View Attachment

D. Travel**Funds Requested (\$)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	20,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	20,000.00

E. Participant/Trainee Support Costs**Funds Requested (\$)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

	Number of Participants/Trainees	Total Participant/Trainee Support Costs	
--	---------------------------------	---	--

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

Start Date: 05/01/2012 * End Date: 04/30/2013 Budget Period

F. Other Direct Costs**Funds Requested (\$)**

1. Materials and Supplies	65,231.00
2. Publication Costs	1,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	642,800.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping	5,000.00
9. Equipment Maintenance	9,000.00
10.	

Total Other Direct Costs 723,031.00**G. Direct Costs****Funds Requested (\$)****Total Direct Costs (A thru F)** 1,291,303.00**H. Indirect Costs**

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. BCM MTDC	56.50	50,000.00	28,250.00
2. BCM-off campus affiliate (TCH) MTDC	57.30	350,000.00	200,550.00
3.			
4.			
Total Indirect Costs			228,800.00

Cognizant Federal Agency DHHS, ARIF KARIM, 214-767-3261

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs**Funds Requested (\$)****Total Direct and Indirect Institutional Costs (G + H)** 1,520,103.00**J. Fee****Funds Requested (\$)****K. * Budget Justification** 1240-FINAL_Budg_Just.pdf

(Only attach one file.)

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View Attachment

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2013 * End Date: 04/30/2014

Budget Period 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.		Peter	J	Hotez	M.D.	PD/PI	Institutional Base Salary	EFFORT			19,970.00	4,593.00	24,563.00
2.		Maria	Elena	Bottazzi	PhD	PD/PI					39,940.00	9,186.00	49,126.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												
												Total Senior/Key Person	73,689.00

Additional Senior Key Persons:

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B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Associate Director for Product Development	EFFORT			19,004.00	5,701.00	24,705.00
1	Director, Process Development				10,031.00	3,009.00	13,040.00
1	Research Scientist for upstream development				4,751.00	1,425.00	6,176.00
1	Research Scientist for downstream development				5,279.00	1,584.00	6,863.00
1	Research Assistant				30,801.00	9,240.00	40,041.00
5	Total Number Other Personnel	Total Other Personnel					90,825.00
Total Salary, Wages and Fringe Benefits (A+B)							164,514.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2013 * End Date: 04/30/2014 Budget Period 2

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

	Equipment item	* Funds Requested (\$)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
Total Equipment		

Additional Equipment:

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D. Travel**Funds Requested (\$)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	10,000.00
2.	Foreign Travel Costs	
Total Travel Cost		10,000.00

E. Participant/Trainee Support Costs**Funds Requested (\$)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

 Number of Participants/Trainees Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

Start Date: 05/01/2013 * End Date: 04/30/2014 Budget Period 2

F. Other Direct Costs

Funds Requested (\$)

1. Materials and Supplies	60,485.00
2. Publication Costs	1,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	798,600.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping	5,000.00
9. Equipment Maintenance	9,000.00
10.	

Total Other Direct Costs 874,085.00

G. Direct Costs

Funds Requested (\$)

Total Direct Costs (A thru F) 1,048,599.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. BCM-off campus affiliate (TCH) MTDC	57.30	249,999.00	143,249.00
2.			
3.			
4.			

Total Indirect Costs 143,249.00

Cognizant Federal Agency DHHS, Arif Karim, 214-767-3261

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

Funds Requested (\$)

Total Direct and Indirect Institutional Costs (G + H)

1,191,848.00

J. Fee

Funds Requested (\$)

K. * Budget Justification 1240-FINAL_Budg_Just.pdf

(Only attach one file.)

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Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2014 * End Date: 04/30/2015 Budget Period 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.		Peter		Hotez		PD/PI	Institutional Base Salary	EFFORT			19,970.00	4,593.00	24,563.00
2.		Maria	Elena	Bottazzi	PhD	PD/PI					29,955.00	6,890.00	36,845.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												
Total Senior/Key Person												61,408.00	

Additional Senior Key Persons:

Add Attachment

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View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Associate Director for Product Development	EFFORT			26,098.00	7,829.00	33,927.00
1	Director, Process Development				20,665.00	6,200.00	26,865.00
1	Research Scientist for Molecular Biology				6,160.00	1,848.00	8,008.00
1	Research Scientist for Upstream Development				24,467.00	7,340.00	31,807.00
1	Research Scientist for Downstream Development				27,186.00	8,156.00	35,342.00
1	Research Assistant				31,725.00	9,518.00	41,243.00
6	Total Number Other Personnel	Total Other Personnel					177,192.00
Total Salary, Wages and Fringe Benefits (A+B)							238,600.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry * Start Date: 05/01/2014 * End Date: 04/30/2015 Budget Period 3

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

	Equipment item	* Funds Requested (\$)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

Funds Requested (\$)

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	20,000.00
2.	Foreign Travel Costs	
	Total Travel Cost	20,000.00

E. Participant/Trainee Support Costs

Funds Requested (\$)

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Close Form

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

Start Date: 05/01/2014 * End Date: 04/30/2015 Budget Period 3

F. Other Direct Costs

Funds Requested (\$)

1. Materials and Supplies	71,400.00
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	516,850.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping	5,000.00
9. Equipment Maintenance	10,000.00
10. Fee-for-service: Sabin	75,000.00

Total Other Direct Costs 683,250.00

G. Direct Costs

Funds Requested (\$)

Total Direct Costs (A thru F) 941,850.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. BCM-off campus affiliate (TCH) MTDC	57.30	425,000.00	243,525.00
2.			
3.			
4.			
Total Indirect Costs			243,525.00

Cognizant Federal Agency DHHS, Arif Karim, 214-767-3261

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

Funds Requested (\$)

Total Direct and Indirect Institutional Costs (G + H)

1,185,375.00

J. Fee

Funds Requested (\$)

K. * Budget Justification 1240-FINAL_Budg_Just.pdf

(Only attach one file.)

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Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2015 * End Date: 04/30/2016

Budget Period 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.		Peter	J	Hotez	M.D.	PD/PI	Institutional Base Salary	EFFORT			19,970.00	4,593.00	24,563.00
2.		Maria	Elena	Bottazzi	PhD	PD/PI					19,970.00	4,593.00	24,563.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												
												Total Senior/Key Person	49,126.00

Additional Senior Key Persons:

Add Attachment

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View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Associate Director for Product Development	EFFORT			6,720.00	2,016.00	8,736.00
1	Director, Process Development				10,642.00	3,193.00	13,835.00
1	Research Assistant				6,535.00	1,961.00	8,496.00
3	Total Number Other Personnel	Total Other Personnel					31,067.00
Total Salary, Wages and Fringe Benefits (A+B)							80,193.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2015 * End Date: 04/30/2016 Budget Period 4

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

	Equipment item	* Funds Requested (\$)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment:

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View Attachment

D. Travel**Funds Requested (\$)**

1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	5,000.00

E. Participant/Trainee Support Costs**Funds Requested (\$)**

1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	

 Number of Participants/Trainees **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

Start Date: 05/01/2015 * End Date: 04/30/2016 Budget Period 4

F. Other Direct Costs**Funds Requested (\$)**

1. Materials and Supplies	10,000.00
2. Publication Costs	807.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	123,150.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping	4,000.00
9. Equipment Maintenance	0.00
10. Fee-for-service: Sabin	575,000.00

Total Other Direct Costs 712,957.00**G. Direct Costs****Funds Requested (\$)****Total Direct Costs (A thru F)** 798,150.00**H. Indirect Costs**

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. BCM-off campus affiliate (TCH) MTDC	57.30	675,000.00	386,775.00
2.			
3.			
4.			

Total Indirect Costs 386,775.00**Cognizant Federal Agency** DHHS, Arif Karim, 214-767-3261

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs**Funds Requested (\$)****Total Direct and Indirect Institutional Costs (G + H)** 1,184,925.00**J. Fee****Funds Requested (\$)****K. * Budget Justification** 1240-FINAL_Budg_Just.pdf

(Only attach one file.)

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Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2016

* End Date: 04/30/2017

Budget Period 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.		Peter	J	Hotez	M.D.	PD/PI	Institutional Base Salary	EFFORT			19,970.00	4,593.00	24,563.00
2.		Maria	Elena	Bottazzi	PhD	PD/PI					19,970.00	4,593.00	24,563.00
3.													
4.													
5.													
6.													
7.													
8.													
9.	Total Funds requested for all Senior Key Persons in the attached file												
												Total Senior/Key Person	49,126.00

Additional Senior Key Persons:

Add Attachment

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View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Associate Director for Product Development	EFFORT			13,844.00	4,153.00	17,997.00
1	Research Assistant				6,731.00	2,019.00	8,750.00
2	Total Number Other Personnel	Total Other Personnel					26,747.00
Total Salary, Wages and Fringe Benefits (A+B)							75,873.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

* Start Date: 05/01/2016 * End Date: 04/30/2017 Budget Period 5

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

	Equipment item	* Funds Requested (\$)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment:

Add Attachment

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D. Travel**Funds Requested (\$)**

1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	5,000.00

E. Participant/Trainee Support Costs**Funds Requested (\$)**

1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	

 Number of Participants/Trainees **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0511133300000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Baylor College of Medicine

Delete Entry

Start Date: 05/01/2016 * End Date: 04/30/2017 Budget Period 5

F. Other Direct Costs**Funds Requested (\$)**

1. Materials and Supplies	10,126.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	82,100.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping	3,000.00
9. Equipment Maintenance	3,000.00
10. Fee-for-Service: Sabin	600,000.00

Total Other Direct Costs 701,226.00**G. Direct Costs****Funds Requested (\$)****Total Direct Costs (A thru F)** 782,099.00**H. Indirect Costs**

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. BCM-off campus affiliate (TCH) MTDC	57.30	699,999.00	401,099.00
2.			
3.			
4.			

Total Indirect Costs 401,099.00**Cognizant Federal Agency** DHHS, Arif Karim, 214-767-3261

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs**Funds Requested (\$)****Total Direct and Indirect Institutional Costs (G + H)**

1,183,198.00

J. Fee**Funds Requested (\$)****K. * Budget Justification** 1240-FINAL_Budg_Just.pdf

(Only attach one file.)

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BUDGET JUSTIFICATION

Baylor College of Medicine

Personnel

Peter J. Hotez, MD, PhD – PD/PI (Effort = [EFFORT] months for years 1-5) has more than 20 years of experience in the development of control tools (i.e vaccines) against neglected tropical diseases. He currently serves as the Director and Principal Scientist of the Vaccine Center at BCM. Dr. Hotez will be responsible for the scientific direction, management and implementation of activities to aim at the final goal of successful and productive running this proposal. Dr. Hotez will encourage and facilitate the timely analysis and submission of data.

Maria Elena Bottazzi, PhD – PD/PI (Effort = [EFFORT] months for years 1-2, [EFFORT] months for year 3 and [EFFORT] months for years 4-5). Dr. Bottazzi has over 11 years of molecular/cellular biology experience and has served for the last 10 years as the Program Director for Product Development of the Vaccine Center. She has ample with expertise in managing complex programs and technical units for the production of recombinant biologics using the bacterial and yeast expression system. She will supervise all operational and programmatic activities of the project, manage timelines and Go/NoGo criteria and decision points in collaboration with Dr Hotez and the PIs from NYBC and UTMB.

Bin Zhan, MD, MS – Co-Investigator (Effort = [EFFORT] months for Year 1) serves as the Director of the Molecular Biology Unit. He will be responsible for all the molecular biology and genetic engineering. With Dr Bottazzi and in collaboration with NYBC, he will coordinate the efforts for the optimization of the cloning into yeast expression system.

Portia Gillespie - Research Scientist for molecular biology (Effort = [EFFORT] months for Year 1 and [EFFORT] months for Year 3) will assist Drs. Zhan, Bottazzi and Hotez in the molecular and feasibility of expression of the recombinant vaccine candidate, screen for expressing clones, prepare a research cell bank of a selected clone, provide initial characterization of secreted proteins and purification of the recombinant proteins. In addition in Year 3 she will be in charge of any stability assays for the research cell bank as well as technology transfer.

Jordan Plieskatt - Associate Director for Product Development (Effort = [EFFORT] months for year 1, [EFFORT] months for Year 2, [EFFORT] months for Year 3, [EFFORT] months for Year 4 and [EFFORT] months for year 5). He will be responsible for organizing the activities for the fermentation, purification processes and the analytical and formulation activities at low and mid scales. With Dr Bottazzi he will coordinate the efforts for the optimization, development and scale-up processes and final process development.

Vehid Deumic, MSc. - Director, Process Development (Effort = [EFFORT] months for year 1, [EFFORT] months for Year 2, [EFFORT] months for Year 3, and [EFFORT] months for Year 4). Mr. Deumic will lead and manage a team involved in research and upstream and downstream process development. These activities include performing design of experiments to improve the production process of the SARS vaccine candidate.

Jill Brelsford – Research Scientist for upstream development (Effort = [EFFORT] months for year 2; [EFFORT] months for year 3) will be responsible for the daily operational and technical work for the fermentation or upstream development activities.

Clifford Kwittin– Research Scientist for downstream development (Effort = [EFFORT] months for year 2; [EFFORT] months for year 3) will be responsible for the daily operational and technical work for the downstream process development and purification activities.

Wanderson Rezende – Research Assistant for biochemical/biophysical and formulation characterization (Effort = [EFFORT] months for Years 1-3 and [EFFORT] months for years 4-5). Mr

Rezende has extensive experience in analytical and biophysical assay development for recombinant vaccines. In addition he will handle the daily activities for formulation development and will work with Dr Bottazzi and Jordan Plieskatt to perform the assays and quality control testing of in processes, release and stability.

Fringe Benefits. Baylor Pediatrics calculates fringe benefits at an average rate of 23% for faculty and 30% for staff.

Equipment

A total of \$298,503 (year 1 only) is requested for equipment as specified below:

Floor model refrigerated incubated shaker. Lab Companion (\$9600) Floor model shaker for expanded temperature range of flask cultures. Would allow for the setting of various temperatures and enhanced throughput of the molecular biology strain screening.

Sample application pump, Air Sensor and Buffer Selection kit for Akta Purifier. GE (\$13,025) Allow for the loading of large volumes of samples on the Akta 10 increasing throughput and expanding sample application volume range. Air sensor upgrade for Akta 10, allowing for more automated processes and risk management. Buffer Selection kit allowing for expanded automated buffer screening.

Circular Dichroism Chiral Detector w/software. Jasco (\$ 31,158) Detector that can be used on or offline Liquid chromatography methods to measure CD signal of samples including structural and conformational analysis of molecules and candidates during development

Mobius with DLS (Zeta potential instrument with dynamic light scattering module) Wyatt (\$ 62, 475) Can measure physical and chemical properties of proteins by electrophoretic mobility. Allows for accessing of three parameters simultaneously at a wide range of molecular weight and pI

Acquity Fluorescence Detector Waters (\$ 14,832) Online fluorescence detector the Acquity H class UPLC, expanding the range of assays and detector capabilities with method development

Parallel Bioreactor System DAS GIP Technology (\$ 140,000) Using DASGIP Parallel Bioreactor Systems scientists benefit from an advanced bioreactor technology gaining superior results in strain characterization, process development and gene expression. DASGIP systems combine the conveniences of simple systems such as shaking flasks with benefits of large-scale bioreactors: Small working volumes allow high experimental throughput with minimal input. Precision and comprehensive process control result in highly scalable and reproducible output. Users working with E. coli, Pichia pastoris, yeast and fungi have experienced the DASGIP system as customized and highly efficient biological processing tool.

Biochemistry Analyzers YSI Life Sciences (\$ 13,818) The YSI 2700 SELECT Biochemistry Analyzer provides vital information about processes. It provides rapid, accurate analysis of key nutrients and byproducts, including: Glucose, Lactate, Glutamine, Glutamate, Ethanol, Lactose, Sucrose, Galactose, Hydrogen peroxide, Methanol, Starch, Choline, Xylose

Spectrophotometer GE (\$ 8,513) Provide an updated spectrophotometer dedicated to process development activities.

Akta Explorer High Flow Kit GE (\$ 5,082) The ÄKTA™ High Flow kit (no. 18-1154-85) is intended for use in ÄKTA explorer 100 systems. The kit contains the necessary components and software for reducing the back-pressure in the system and thereby allowing higher flow rates. This makes it possible to use the system for larger columns and by that increasing the production capacity.

Materials and Lab Supplies

Materials and supplies requested of \$217,242 for 5 years include those necessary for molecular biology, protein expression and purification, analytical and biochemical analysis such as media, chemicals, resins, buffers, and glassware and pipettes, plastics and disposables. Supplies include reagents such as media,

chemicals, resins, antibodies, buffers, LAL kits, purification columns membranes and glassware and pipettes, plastics and disposables in order to perform the scale up production.

Travel. We request a total of \$ 60,000 for a period of five years for travel for the PIs and senior scientists to travel to a scientific meeting in order to present findings at national conferences. In addition, visits to discuss the project with NYBC and UTMB can be coordinated.

Publications/slides/posters We request a total of \$10,807 for publications and any other needs for presentations during meetings and conferences

Shipping. We request a total of \$22,000 for the period of five years to cover shipment of biologics, other samples and related costs.

Equipment Maintenance: \$31,000 is requested for years 1-5 for maintaining equipments used for this grant.

Fee-for-service contractor: The fee-for-service contract with **Sabin Vaccine Institute** will be distributed as follows: Year 3: \$75,000; Year 4: \$575,000 and Year 5: \$600,000; a total of \$1,250,000 for yrs 3-5. More information is provided under the "Contractual Arrangements" section.

Sub-awards: **New York Blood Center** and **University of Texas Medical Branch.**

The allocations for these sub-awards are as follows:

Organization	Direct Costs per year				
	Year 1	Year 2	Year 3	Year 4	Year 5
NYBC	\$275,000	\$ 300,000	\$ 175,000	\$ 75,000	\$ 50,000
UTMB	\$125,000	\$ 200,000	\$ 150,000	\$ 0	\$ 0

Separate budget justification for each sub-award is provided as an attachment to their own budget. Further information is provided under the "Contractual Arrangements" section.

Facilities and Administrative Costs (Indirect Costs). Indirect costs are calculated using the off-campus Baylor rate of 27.1% combined with the Texas Children's Hospital affiliated rate of 30.2% for a combination rate of 57.3% for work performed at Texas Children's Hospital. Indirect costs are also being taken on the first \$25,000 of each subcontract utilizing the Baylor on-campus rate of 56.5% per the DHHS rate agreement.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals (\$)
Section A, Senior/Key Person		322,451.00
Section B, Other Personnel		486,498.00
Total Number Other Personnel	20	
Total Salary, Wages and Fringe Benefits (A+B)		808,949.00
Section C, Equipment		298,503.00
Section D, Travel		60,000.00
1. Domestic	60,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		3,694,549.00
1. Materials and Supplies	217,242.00	
2. Publication Costs	10,807.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	2,163,500.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	22,000.00	
9. Other 2	31,000.00	
10. Other 3	1,250,000.00	
Section G, Direct Costs (A thru F)		4,862,001.00
Section H, Indirect Costs		1,403,448.00
Section I, Total Direct and Indirect Costs (G + H)		6,265,449.00
Section J, Fee		

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2012* **End Date:** 04-30-2013**Budget Period:** 1**A. Senior/Key Person**

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.	Dr.	Shibo	Jiang	MD	PD/PI	Institutional Base Salary	EFFORT			48,104.00	15,874.00	63,978.00
2.	Dr.	Sara	Lustigman	PhD	Co-investigator					17,293.00	5,706.00	22,999.00
3.	Dr.	Lanying	Du	PhD	Co-investigator					18,865.00	6,225.00	25,090.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:			File Name:		Mime Type:		Total Senior/Key Person				112,067.00	

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	EFFORT			18,791.00	6,201.00	24,992.00
1	Research Fellow				38,000.00	12,540.00	50,540.00
2	Total Number Other Personnel					Total Other Personnel	75,532.00
					Total Salary, Wages and Fringe Benefits (A+B)		187,599.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2012* **End Date:** 04-30-2013**Budget Period:** 1

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	8,000.00
2. Foreign Travel Costs	
Total Travel Cost	8,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2012* **End Date:** 04-30-2013**Budget Period:** 1

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	51,000.00
2. Publication Costs	1,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	5,000.00
7. Alterations and Renovations	
8. Animal Purchase & Maintenance	20,000.00
9. Miscellaneous	2,401.00
Total Other Direct Costs	79,401.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	275,000.00

H. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. MTDC		64.20	275,000.00	176,550.00
			Total Indirect Costs	176,550.00
Cognizant Federal Agency		DHHS, Jeffrey Warren, 212-264-2069		
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	451,550.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name: 1247-Budget justification	Mime Type: application/pdf
	NYBC_final.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2013* **End Date:** 04-30-2014**Budget Period:** 2**A. Senior/Key Person**

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.	Dr.	Shibo	Jiang	MD	PD/PI	Institutional Base Salary	EFFORT			49,547.00	16,351.00	65,898.00
2.	Dr.	Sara	Lustigman	PhD	Co-investigator					17,811.00	5,878.00	23,689.00
3.	Dr.	Lanying	Du	PhD	Co-investigator					19,430.00	6,412.00	25,842.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:			File Name:		Mime Type:		Total Senior/Key Person					115,429.00

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	EFFORT			19,354.00	6,387.00	25,741.00
1	Research Fellow				41,000.00	13,530.00	54,530.00
2	Total Number Other Personnel					Total Other Personnel	
		Total Salary, Wages and Fringe Benefits (A+B)					195,700.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2013* **End Date:** 04-30-2014**Budget Period:** 2

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	8,000.00
2. Foreign Travel Costs	
Total Travel Cost	8,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2013* **End Date:** 04-30-2014**Budget Period:** 2

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	52,000.00
2. Publication Costs	1,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	5,000.00
7. Alterations and Renovations	
8. Animal purchase and maintenance	35,000.00
9. Miscellaneous	3,300.00
Total Other Direct Costs	96,300.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	300,000.00

H. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. MTDC		64.20	300,000.00	192,600.00
			Total Indirect Costs	192,600.00
Cognizant Federal Agency		DHHS, Jeffrey Warren, 212-264-2069		
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	492,600.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name: 1247-Budget justification	Mime Type: application/pdf
	NYBC_final.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2014* **End Date:** 04-30-2015**Budget Period:** 3**A. Senior/Key Person**

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.	Dr.	Shibo	Jiang	MD	PD/PI	Institutional Base Salary	EFFORT			29,955.00	9,885.00	39,840.00
2.	Dr.	Sara	Lustigman	PhD	Co-investigator					9,173.00	3,027.00	12,200.00
3.	Dr.	Lanying	Du	PhD	Co-investigator					6,671.00	2,201.00	8,872.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:			File Name:		Mime Type:		Total Senior/Key Person					60,912.00

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant	EFFORT			19,935.00	6,579.00	26,514.00
1	Research Fellow				22,500.00	7,425.00	29,925.00
2	Total Number Other Personnel					Total Other Personnel	
					Total Salary, Wages and Fringe Benefits (A+B)		117,351.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2014* **End Date:** 04-30-2015**Budget Period:** 3

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	8,000.00
2. Foreign Travel Costs	
Total Travel Cost	8,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2014* **End Date:** 04-30-2015**Budget Period:** 3

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	36,000.00
2. Publication Costs	1,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	5,000.00
7. Alterations and Renovations	
8. Animal Purchase and maintenance	5,000.00
9. Miscellaneous	2,649.00
Total Other Direct Costs	49,649.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	175,000.00

H. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. MTDC		64.20	175,000.00	112,350.00
			Total Indirect Costs	112,350.00
Cognizant Federal Agency		DHHS, Jeffrey Warren, 212-264-2069		
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	287,350.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name: 1247-Budget justification	Mime Type: application/pdf
	NYBC_final.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2015* **End Date:** 04-30-2016**Budget Period:** 4**A. Senior/Key Person**

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary	Cal.	Acad.	Sum.	* Requested	* Fringe	* Funds Requested (\$)
						(\$)	Months	Months	Months	Salary (\$)	Benefits (\$)	
1.	Dr.	Shibo	Jiang	MD	PD/PI	Institutional	EFFORT			29,955.00	9,885.00	39,840.00
2.	Dr.	Sara	Lustigman	PhD	Co-investigator	Base Salary				9,448.00	3,118.00	12,566.00
3.	Dr.	Lanying	Du	PhD	Co-investigator					3,435.00	1,134.00	4,569.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:			File Name:		Mime Type:		Total Senior/Key Person					56,975.00

B. Other Personnel

* Number of Personnel	* Project Role	Cal.	Acad.	Sum.	* Requested	* Fringe	* Funds Requested
		Months	Months	Months	Salary (\$)	Benefits	(\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel						
Total Other Personnel							
Total Salary, Wages and Fringe Benefits (A+B)							56,975.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2015* **End Date:** 04-30-2016**Budget Period:** 4

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	4,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2015* **End Date:** 04-30-2016**Budget Period:** 4

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	8,000.00
2. Publication Costs	1,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	3,000.00
7. Alterations and Renovations	
8. Miscellaneous	2,025.00
Total Other Direct Costs	14,025.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	75,000.00

H. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. MTDC		64.20	75,000.00	48,150.00
			Total Indirect Costs	48,150.00
Cognizant Federal Agency		DHHS, Jeffrey Warren, 212-264-2069		
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	123,150.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name: 1247-Budget justification	Mime Type: application/pdf
	NYBC_final.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2016* **End Date:** 04-30-2017**Budget Period:** 5**A. Senior/Key Person**

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.	Dr.	Shibo	Jiang	MD	PD/PI	Institutional Base Salary	EFFORT			29,955.00	9,885.00	39,840.00
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:			File Name:		Mime Type:		Total Senior/Key Person					39,840.00

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Total Number Other Personnel	Total Other Personnel					
Total Salary, Wages and Fringe Benefits (A+B)							39,840.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2016* **End Date:** 04-30-2017**Budget Period:** 5

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	4,000.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5* **ORGANIZATIONAL DUNS:** 0732718270000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** New York Blood Center* **Start Date:** 05-01-2016* **End Date:** 04-30-2017**Budget Period:** 5

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	
2. Publication Costs	1,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	3,000.00
7. Alterations and Renovations	
8. Miscellaneous	2,160.00
Total Other Direct Costs	6,160.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	50,000.00

H. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. MTDC		64.20	50,000.00	32,100.00
			Total Indirect Costs	32,100.00
Cognizant Federal Agency		DHHS, Jeffrey Warren, 212-264-2069		
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	82,100.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name: 1247-Budget justification	Mime Type: application/pdf
	NYBC_final.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)	
Section A, Senior/Key Person		385,223.00
Section B, Other Personnel		212,242.00
Total Number Other Personnel	6	
Total Salary, Wages and Fringe Benefits (A+B)		597,465.00
Section C, Equipment		
Section D, Travel		32,000.00
1. Domestic	32,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		245,535.00
1. Materials and Supplies	147,000.00	
2. Publication Costs	5,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees	21,000.00	
7. Alterations and Renovations		
8. Other 1	64,185.00	
9. Other 2	8,350.00	
10. Other 3		
Section G, Direct Costs (A thru F)		875,000.00
Section H, Indirect Costs		561,750.00
Section I, Total Direct and Indirect Costs (G + H)		1,436,750.00
Section J, Fee		

BUDGET JUSTIFICATION New York Blood Center**PERSONNEL:**

Shibo Jiang, M.D., Member, PI#2, Head of Viral Immunology Laboratory, Lindsley F. Kimball Research Institute of New York Blood Center [EFFORT] effort or [EFFORT] months for years 1-2 and [EFFORT] effort or [EFFORT] months for years 3-5), will plan, direct and execute the majority of the studies in Aim 1 in close involvement and discussion with the PI#1, PI#3 and Dr. Sara Lustigman. He will supervise Dr. Lanying Du (Co-investigator) and Dr. Peng Zou (Postdoctoral Research Fellow) and participate in the related experiments, evaluate the experimental results, and the writing of progress reports and papers. He will also be the liaison for the efficacy studies in collaboration with Dr. Chien-Te K. Tseng at the Department of Microbiology and Immunology, University of Texas Medical Branch (UTMB).

Sara Lustigman, Ph.D., Member, Co-Investigator, Head of the Laboratory of Molecular Parasitology, Lindsley F. Kimball Research Institute of New York Blood Center [EFFORT] effort or [EFFORT] months for years 1-2 and [EFFORT] effort or [EFFORT] months for years 3-4), has more than 20 years of post-doctoral experience as a molecular biologist and immunoparasitologist, many of which has been on vaccine development against parasites. She will interact with PI#1, PI#2 and PI#3 and provide her expertise to the research team for development of RBD-based SARS vaccine. In addition, she will be the NYBC liaison for all aspects involving the product development of recombinant RBD and adjuvant formulation for optimization. She will oversee the research conducted by her Research Assistant, Nancy Tricoche.

Lanying Du, Ph.D., Assistant Member, Co-Investigator, Viral Immunology Laboratory, Lindsley F. Kimball Research Institute [EFFORT] effort or [EFFORT] months for years 1-2, [EFFORT] effort or [EFFORT] months for year 3 and [EFFORT] effort or [EFFORT] months for year 4), will prepare pseudoviruses expressing S proteins of homologous and heterologous SARS-CoV and testing the neutralizing and cross-neutralizing antibodies. She will also be responsible for immunization of the mice and for optimization of the vaccination regimens. She will report to Dr. Jiang.

Peng Zou, Ph.D., Postdoctoral Research Fellow, Laboratory of Viral Immunology, Lindsley F. Kimball Research Institute [EFFORT] effort or [EFFORT] months for years 1-2 and [EFFORT] effort or [EFFORT] months for year 3), will be responsible for performing ELISA for measuring the antigenicity using RBD-specific and confirmation-dependent mAbs, testing the functionality of rRBD protein using ELISA or ITC for measuring binding of rRBD protein to soluble ACE2 or flow cytometry for detecting the binding of rRBD protein to ACE2-expressing cells, and assessing the immunogenicity of rRBD proteins using ELISA for determining the titers of the RBD-specific antibodies, including IgG1, IgG2a, IgG2b, IgG3 and IgA. He will report to Dr. Jiang.

Nancy Tricoche, Research Assistant III, Laboratory of Molecular Parasitology, Lindsley F. Kimball Research Institute of New York Blood Center [EFFORT] effort or [EFFORT] months for years 1-3), will be responsible for testing the immunogenicity of rRBD protein in regard to all cellular responses including the induction of MHC-H-2d restricted SARS-CoVspecific cytotoxic T lymphocyte response by using ELISPOT and other alternative assays. She will report to Dr. Lustigman.

TRAVEL:

Travel expense (\$8,000 for years 1-3 and \$4,000 for years 4-5) is requested for Drs. Lustigman, Jiang, Du and Zou to attend one scientific conference each year and for Dr. Jiang and/or Dr. Lustigman to travel to an annual meeting in Bethesda, Maryland, or to a relevant scientific meeting, as determined by NIAID Program staff, and one trip to Texas for meeting with the PI#1 and PI#3 and their groups.

OTHER DIRECT COSTS:

MATERIALS & SUPPLIES (total \$147,000: \$51,000 for year 1; \$52,000 for year 2; \$36,000 for year 3; and \$8,000 for year 4)

Tissue culture media (\$34,000): A large amount of cell cultures will be carried out for testing the rRBD-induced immune responses in animal models, including neutralizing and cross-neutralizing antibodies against SARS pseudoviruses, T cell responses and cytotoxic T cell activity. Therefore, about \$9,000, \$12,000, \$8,000 and \$5000 for years 1 to 4, respectively, are requested for purchasing cell culture media and FCS sera.

Plastic and glassware (\$29,000): About \$9,000, \$9,000, \$8000 and \$3000 for years 1 to 4, respectively, are requested for purchasing plastic and glassware, including tissue culture flasks, 96 well plates, disposable tubes, pipettes, tips, etc.

General Lab supplies (\$22,000): About \$6,000, \$8000, \$5000 and \$3000 for years 1 to 4, respectively, are requested for purchasing chemicals, buffers, antibiotics, cryovials, gloves, gowns, and miscellaneous supplies.

Antibodies and antibody conjugates (\$27,000): For testing the antigenicity, functionality, and immunogenicity of rRBD proteins, a number of antibodies and second antibodies will be needed for the ELISA and FACS analysis. About \$7,000, \$10,000, \$6000 and \$4000 for years 1 to 4, respectively, are requested to purchasing the labeled and unlabeled antibodies.

Reagents for preparing SARS pseudovirus and for neutralizing assays (\$35,000): About \$9,000, \$13,000, 9,000 and \$4000 for years 1 to 4, respectively, are requested to purchasing reagents for generation of SARS pseudoviruses and the luciferase kits and related reagents for testing the neutralizing and cross-neutralizing antibodies against SARS pseudoviruses.

PUBLICATION COSTS: \$1,000 per year is requested.

EQUIPMENT/FACILITY RENTAL/USE: About \$4,200 per year for the years 1-5 requested for the costs of maintaining equipment (e.g., culture hoods, centrifuges, freezers, CO2 incubators, ELISPOT reader, ELISA reader, ELISA washer, etc.) and the costs of using equipment in NYBC's Core Facility, such as the FACSCanto analyzer.

OTHER EXPENSES:

Animal purchase and maintenance (total \$60,000: \$20,000 for year 1, \$35,000 for year 2, and \$5,000 for year 3): about 40 groups of mice (10 mice/group) will be used for the studies in Aim 1, including 10 groups for immunogenicity study in Aim 1.C, 12 groups for optimization of antigen dose and adjuvant formulation in Aim 1.E.1 (see TABLE II in Milestone), and 18 groups for optimization of route of administration and dose frequency of immunizations in Aim 1.E.2 (see TABLE III in Milestone). Each experiment will be repeated twice. Therefore, total about 1200 mice will be needed. The maintenance period for each adult mouse is about 90 days. The cost for purchase is \$20.45/mouse and for maintenance is \$0.32/day/mouse.

Miscellaneous (\$12,534): About \$2,500 per year for the years 1-5 is requested for Misc. expenses, such as office supplies, communication, shipping, and printing expenses, etc.

*Year 2-5 increases: Salaries are increased at the rate of 3% per year. The NIH salary cap for 2011 is \$199,700.

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2012* **End Date:** 04-30-2013**Budget Period:** 1**A. Senior/Key Person**

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.	Dr.	Chien-te	Kent	Tseng	PhD	PD/PI	Institutional Base Salary	EFFORT			24,399.00	5,809.00	30,208.00
2.	Dr.	Tehsheng		Chan	PhD	Co-Investigator					5,717.00	1,219.00	6,936.00
Total Funds Requested for all Senior Key Persons in the attached file													
Additional Senior Key Persons:				File Name:			Mime Type:			Total Senior/Key Person			37,144.00

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	EFFORT			20,500.00	6,494.00	26,994.00
1	Research Assistant				17,340.00	5,493.00	22,833.00
2	Total Number Other Personnel					Total Other Personnel	
					Total Salary, Wages and Fringe Benefits (A+B)		86,971.00

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2012* **End Date:** 04-30-2013**Budget Period:** 1

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	
Total Travel Cost	1,500.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2012* **End Date:** 04-30-2013**Budget Period:** 1

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	27,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal care and maintenance	3,000.00
9. Core Charges (Bioplex, histology, pathology)	6,529.00
Total Other Direct Costs	36,529.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	125,000.00

H. Indirect Costs				
Indirect Cost Type		Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. Modified total direct costs		53.00	125,000.00	66,250.00
			Total Indirect Costs	66,250.00
Cognizant Federal Agency		DHHS Division of Cost Allocation, Darryl Mayes, 202-401-2808		
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	191,250.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name:	Mime Type: application/pdf
	1248-UTMBBUDGETJUSTIFICATION.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2013* **End Date:** 04-30-2014**Budget Period:** 2**A. Senior/Key Person**

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.	Dr.	Chien-te	Kent	Tseng	PhD	PD/PI	Institutional Base Salary	EFFORT			29,864.00	7,111.00	36,975.00
2.	Dr.	Tehsheng		Chan	PhD	Co-investigator					5,831.00	1,244.00	7,075.00
Total Funds Requested for all Senior Key Persons in the attached file													
Additional Senior Key Persons:				File Name:			Mime Type:			Total Senior/Key Person			44,050.00

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	EFFORT			20,910.00	6,624.00	27,534.00
1	Research Assistant				35,374.00	11,206.00	46,580.00
2	Total Number Other Personnel					Total Other Personnel	
					Total Salary, Wages and Fringe Benefits (A+B)		118,164.00

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2013* **End Date:** 04-30-2014**Budget Period:** 2

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	
Total Travel Cost	1,500.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2013* **End Date:** 04-30-2014**Budget Period:** 2

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	65,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal maintenance	7,000.00
9. Core Charges (Bioplex, Histology, Pathology)	8,336.00
Total Other Direct Costs	80,336.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	200,000.00

H. Indirect Costs				
Indirect Cost Type		Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. Modified total direct costs		53.00	200,000.00	106,000.00
			Total Indirect Costs	106,000.00
Cognizant Federal Agency		DHHS Division of Cost Allocation, Darryl Mayes, 202-401-2808		
(Agency Name, POC Name, and POC Phone Number)				

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	306,000.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name:	Mime Type: application/pdf
	1248-UTMBBUDGETJUSTIFICATION.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2014* **End Date:** 04-30-2015**Budget Period:** 3**A. Senior/Key Person**

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$)
1.	Dr.	Chien-te	Kent	Tseng	PhD	PD/PI		EFFORT			30,461.00	7,253.00	37,714.00
2.	Dr.	Tehsheng		Chan	PhD	Co-investigator					5,947.00	1,269.00	7,216.00

Total Funds Requested for all Senior Key Persons in the attached file**Additional Senior Key Persons:**

File Name:

Mime Type:

Total Senior/Key Person**44,930.00****B. Other Personnel**

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary (\$)	* Fringe Benefits	* Funds Requested (\$)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate				21,328.00	6,757.00	28,085.00
1	Research Assistant				36,081.00	11,430.00	47,511.00
2	Total Number Other Personnel					Total Other Personnel	75,596.00
Total Salary, Wages and Fringe Benefits (A+B)							120,526.00

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2014* **End Date:** 04-30-2015**Budget Period:** 3

C. Equipment Description		
List items and dollar amount for each item exceeding \$5,000		
Equipment Item		* Funds Requested (\$)
Total funds requested for all equipment listed in the attached file		
Total Equipment		
Additional Equipment:	File Name:	Mime Type:

D. Travel	Funds Requested (\$)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,500.00
2. Foreign Travel Costs	
Total Travel Cost	1,500.00

E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	Total Participant/Trainee Support Costs

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3* **ORGANIZATIONAL DUNS:** 8007711490000* **Budget Type:** ☐ Project ☒ Subaward/Consortium**Enter name of Organization:** The University of Texas Medical Branch* **Start Date:** 05-01-2014* **End Date:** 04-30-2015**Budget Period:** 3

F. Other Direct Costs	Funds Requested (\$)
1. Materials and Supplies	22,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal maintenance	3,974.00
9. Core Charges (Bioplex, Histology, Pathology)	2,000.00
Total Other Direct Costs	27,974.00

G. Direct Costs	Funds Requested (\$)
Total Direct Costs (A thru F)	150,000.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1. Modified total direct	53.00	150,000.00	79,500.00
		Total Indirect Costs	79,500.00
Cognizant Federal Agency			
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)
Total Direct and Indirect Institutional Costs (G + H)	229,500.00

J. Fee	Funds Requested (\$)
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K. * Budget Justification	File Name:	Mime Type: application/pdf
	1248-UTMBBUDGETJUSTIFICATION.pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals (\$)	
Section A, Senior/Key Person		126,124.00
Section B, Other Personnel		199,537.00
Total Number Other Personnel	6	
Total Salary, Wages and Fringe Benefits (A+B)		325,661.00
Section C, Equipment		
Section D, Travel		4,500.00
1. Domestic	4,500.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		144,839.00
1. Materials and Supplies	114,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	13,974.00	
9. Other 2	16,865.00	
10. Other 3		
Section G, Direct Costs (A thru F)		475,000.00
Section H, Indirect Costs		251,750.00
Section I, Total Direct and Indirect Costs (G + H)		726,750.00
Section J, Fee		

BUDGET JUSTIFICATION

PERSONNEL:

The UTMB-Galveston fringe benefit rate is calculated by salary range as follows:

Salary range	Fringe benefit rate	Salary range	Fringe benefit rate
\$1 - \$65,000	31.68%	\$140,101 - \$180,000	19.53%
\$65,001 - \$79,100	25.31%	\$180,001 - \$245,000	17.95%
\$79,101 - \$106,800	23.81%	\$245,001 - +	14.32%
\$106,801 - \$140,000	21.33%		

Principal Investigator: Chien-Te K. Tseng, PhD, Associate Professor, Department of Microbiology and Immunology, Center for Biodefense and Emerging Diseases, Institute of Human Infection and Immunity, Sealy Center for Vaccine Development, and Galveston National Laboratory (GNL) at UTMB-Galveston, will serve as principal investigator. Dr. Tseng's main research interest focuses on defining the host innate antiviral responses against and the pathogenesis of emerging and reemerging RNA viruses, including severe acute respiratory syndrome (SARS) coronavirus (CoV), Rift Valley Fever (RVF) virus, Dhori virus (a surrogate for avian influenza H5N1 virus), and Junin virus. Dr. Tseng has been a lead investigator in SARS pathogenesis ever since the initial outbreak of SARS in early 2003. In addition to his well-accepted *in vitro* model for SARS-CoV-host interaction by using pathologically relevant human lung epithelial cells, he is a lead author in establishing transgenic mice expressing human angiotensin-converting enzyme 2 (hACE2), the functional viral receptor, shown to be highly susceptible to diseases and mortality associated with SARS-CoV infection. He has also extended his animal models beyond the transgenic mouse model for his SARS research, including several strains of gene knockout (KO) mice and wild-type (*wt*) mice. He also has adopted a mouse-adapted strain of SARS-CoV, termed MA-15, which causes lethal infection in otherwise resistant *wt* BALB/c mice. His highly dedicated team for this proposed project consists of two highly trained and experienced research associates and is fully capable of evaluating the efficacy of candidate vaccines. Dr. Tseng will be fully engaged in all phases of this project, including coordinating, planning and designing, and together with his staff, executing and supervising the proposed experiments. He will also be responsible for the interpretation of the data, preparing the reports and manuscripts as required for publications. He will devote effort (months) in year 1, and effort (months) in Years 2 and 3 to the project.

Co-Investigator: Tehsheng Chan, MD/PhD, Professor, Department of Microbiology and Immunology. Dr. Chan, an accomplished geneticist and cell biologist, joined the UTMB SARS research group consisting of virologists and immunologists soon after the SARS epidemic broke out. One of his major contributions to the group is to design and develop transgenic mouse models for SARS which led to the derivation of mouse models for lethal and non-lethal infection by SARS-CoV. One of the transgenic mouse lineages will be a key model to be used in this contract research. Dr. Chan's past experience included continuous NIH-supported research, including R01, R21 and R03, as well as a SBRI grant and a contract, since 1973. Dr. Chan will be responsible for the maintenance and production of genetically verified, phenotypically stable, pathogen-free transgenic mice for the proposed experiments. He will devote effort (months) for years 1-3 to the project.

Research Staff: Patrick Newman, Research Associate, has been working with Dr. Tseng on SARS-animal models for the last 8 years. He is one the most best-trained research staff members with high containment laboratory experience, including A/BSL-3 and A/BSL-3E, and A/BSL-4. His primary duty will be to carry out the ABSL-3 experiment, including inoculation, monitoring infected animals, collecting various tissues for various analyses (e.g., virologic, immunological, and histopathological changes). He will devote effort (months) for years 1-3 to the project.

Research Staff: Tania Garron, Research Associate, has also been working with Dr. Tseng on SARS research for the last 6+ years. In addition to teaming up with Patrick Newman for carrying out the animal studies, she will be working with the PI in conducting laboratory analyses required to complete the proposed studies, including titrating virus, preparing tissues for histology, immunology, cytokine profiling, pathology, performing IHC and

microneutralization tests. She will also assist Dr. Chan in the phenotyping and maintenance of transgenic mouse colonies. In addition, she will be responsible for recordkeeping and maintaining Dr. Tseng's BSL-2 and A/BSL-3 laboratories. She will devote

 effort (

 months) for year 1 and

 effort (

 months) for years 2-3 to the project.

SUPPLIES: Monies have been budgeted for consumable laboratory supplies to perform hematology, clinical chemistry, virology, and pathology assays as outlined in the tables below.

ANIMAL PURCHASE:

Year 1: \$ 1,500 (60 Balb/c mice @ \$24/mouse plus shipping expenses)
Year 2: \$ 12,000 (120 transgenic mice @ \$100/mouse)
Year 3: \$ 6,250 (ca. 250 Balb/c mice @\$24/mouse plus shipping expenses)

TRAVEL: Funds in the amount of \$1,500 per year for years 1-3 have been budgeted for Drs. Tseng and Chan to present results at national meetings.

OTHER EXPENSES:

Funds are requested for core charges (Histology, Pathology, Bioplex) and animal care costs.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: * First Name:
Middle Name:
* Last Name:
Suffix:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes
* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: * First Name:
Middle Name:
* Last Name:
Suffix:
* Phone Number: Fax Number:
Email:

* Title:

* Street1:
Street2:
* City:
County/Parish:
* State:
Province:
* Country: * Zip / Postal Code:

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells?



No



Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: <http://stemcells.nih.gov/research/registry/>. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s):



Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1241-Specific_Aims.pdf	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1242-Research_Strategy_PJH.p	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects	1257-Human_Subjects.pdf	Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities	1258-Inclusion_wom_Min_PJH.p	Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table	1259-Targeted_enrollment.pd	Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children	1260-INCLUSION_OF_CHILDREN.p	Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1261-Vertebrate_animals.pdf	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan	1262-Multiple_PI_Plan_PJH.pd	Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements	1263-Contractual_agreement_	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1264-Support_Letters.pdf	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1265-Resource_Sharing_Plan.p	Add Attachment	Delete Attachment	View Attachment

16. Appendix [Add Attachments](#) [Remove Attachments](#) [View Attachments](#)

Specific Aims

Severe acute respiratory syndrome (SARS), the first emerging infectious disease of the 21st century, can inflict devastating disease outcomes and disrupt the social, economic and political stability on an international scale as it did during the 2002-03 pandemic. Its causative pathogen, SARS coronavirus (SARS-CoV), has been classified by NIAID as a Category C Priority Pathogen. Therefore, development of an effective and safe vaccine for preventing future SARS outbreaks and for biodefense preparedness is urgently needed. In response to RFA-AI-11-014, Baylor College Medicine, the new home of Sabin Vaccine Institute's product development partnership (BCM-Sabin), will link with SARS research groups located at the New York Blood Center (NYBC) and University of Texas Medical Branch Galveston (UTMB) to develop, test and manufacture a novel recombinant SARS vaccine. **The strengths of the proposal include BCM-Sabin's eleven-year track record of advancing recombinant vaccines for neglected tropical diseases through product development, manufacture, regulatory filing, and clinical testing, together with NYBC's extensive body of preliminary data to justify the selection of the proposed candidate vaccine antigen, and UTMB's first transgenic mouse model for SARS-CoV infection.** The NYBC's group is the first to show that the receptor-binding domain (RBD) of SARS-CoV spike (S) protein contains the major neutralizing epitopes and RBD-based vaccine can induce potent neutralizing antibody responses and protection in animals against SARS-CoV infection. Another strength of the proposal is a high level of **innovation**, which include biophysical techniques to optimize product stability (for emergency stockpiling), selection of the RBD portion of the S protein to reduce the risk of immune enhancement, adjuvant access, and testing in a transgenic model of SARS in mice that resembles human disease. For this proposal NYBC will evaluate the antigenicity, functionality, and immunogenicity of the RBD-based SARS vaccine; UTMB will evaluate the *in vivo* efficacy of the RBD-based vaccine; and BCM-Sabin will express the recombinant vaccine and perform process development and technology transfer activities for manufacture, in addition to providing regulatory affairs and quality assurance. As an additional strength BCM-Sabin has enlisted key industrial partners for: 1) cGMP manufacture; 2) access to glucopyranosyl lipid A (GLA), a novel TLR4 adjuvant; and 3) GLP toxicology testing. Ultimately, at the end of the project, all information and processes will be in place for compilation/submission of an IND and entry into clinical development.

Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. We will evaluate the expression of the recombinant RBD (rRBD) in bacteria and yeast expression systems and select one of them for expression of rRBD protein for subsequent studies based on yields, purity, stability, antigenicity, functionality, immunogenicity, and efficacy (for inducing neutralizing antibody responses and protection against SARS-CoV challenge) of the rRBD protein when formulated in alum. We will use the rRBD protein from the selected expression system for optimization of immunization regimens, and assess the ability of rRBD protein formulated with alum based adjuvants and/or GLA, a TLR4 antagonist adjuvant, to induce cross-neutralizing antibody response, cross-protection and long-term immune responses and protection in mouse models using the optimized immunization regimen. (Timeline Year 1-3)

Specific Aim 2: Process development, characterization, formulation and stability profiling. In parallel to Aim 1, a scalable and reproducible fermentation process for rRBD (10 liter scale) and a purification process using chromatographic technologies will be developed. Reproducibility will be confirmed and specific product quality assays will be developed and used to characterize the recombinant vaccine protein. Vaccine buffer formulations will be developed and characterized using an innovative approach, combining analytical/biochemical tools with biophysical assays to test different excipients and stabilizers and establish an optimal stability profile. The stabilized protein will be formulated with alum and/or GLA. The binding and effect on the structure stability will be examined. Immunogenicity and efficacy of the vaccine formulations will be evaluated in parallel as described in Aim 1. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. (Timeline Year 2-4)

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. The cell bank production, production processes and formulation technology for the selected rRBD-based vaccine will be transferred to Walter Reed Army Institute of Research (WRAIR) pilot facility for 60-L scale GMP manufacture, formulation and fill and finish. The clinical lots will be released by BCM-Sabin and following a pre-IND meeting with the U.S. FDA, GLP toxicology will be initiated at Frontier Biosciences, a Maryland-based contractor. BCM-Sabin will prepare and submit an IND in preparation for the initiation of the clinical development plan. (Timeline Year 4-5)

Research Strategy:

(a) Significance

SARS. SARS is considered the first new infectious disease identified in the 21st century and classified by NIAID as a Category C biodefense agent with an intense capacity to inflict devastating disease outcomes and disrupt local, national, and global economies as it did during the 2002-03 pandemic. It originated in Guangdong province of China in November 2002 and ultimately spread along international air-travel routes to and across five continents. This spread led to over 8,000 infections and almost 800 deaths, before the outbreak was contained through an aggressive program of quarantine, patient isolation and travel restrictions. While the overall mortality rate from SARS during the 2002-03 pandemic was approximately 10%, the mortality among the elderly exceeded 50% (1). In April of 2003, a new coronavirus, SARS-CoV, was identified as the etiologic agent, and subsequently shown to be an envelope virus with a single stranded positive-sense RNA genome encoding a nonstructural replicase polyprotein and several structural proteins including a spike (S) and envelope (E) proteins (1). SARS-CoV is a zoonotic virus with the Chinese horseshoe bat and other exotic species (e.g., palm civets, raccoon dogs, etc.) as its putative natural reservoirs. Although SARS-CoV was believed to be eliminated from human circulation in July 2003, its brief recurrences in laboratory workers in China between late 2003 and early 2004, and its continued existence in nature pose a threat for its reemergence in human populations through either zoonotic reintroduction, laboratory escape or when used as a bioterrorism agent. Thus, there is an urgent need for effective strategies against this devastating viral agent especially in high-risk groups including the elderly, healthcare workers and laboratory personnel and for biodefense preparedness (2).

The RBD protein as the vaccine antigen. An important strength of this proposal is our seven years of preliminary data justifying RBD as a key vaccine target. SARS CoV-specific IgG is detectable two weeks post-infection in humans reaching a peak approximately 60 days post-infection. High titers of neutralizing antibodies as well as specific cytotoxic T lymphocyte (CTL) responses are detectable in patients who recover from SARS, with levels correlating with disease outcome (3-6). In laboratory animal models, neutralizing anti-RBD IgG is linked to protective efficacy following virus challenge (1). Neutralizing antibodies and/or CTLs can be raised against several SARS-CoV structural proteins, especially the spike (S) protein, which is responsible for receptor recognition, virus attachment, and entry, thereby suggesting that S protein is a promising target for the development of anti-SARS vaccines (1). The spikes of SARS-CoV are comprised of trimers of the S protein, which belong to a group of class 1 viral fusion glycoproteins, including HIV glycoprotein 160, influenza hemagglutinin, paramyxovirus F, and Ebola glycoprotein. The SARS-CoV S protein encodes a glycoprotein precursor of 1,256 amino acids (aa), with the amino terminus outside of the cell surface or virus particle. The precursor consists of a signal peptide (aa 1-12), an extracellular domain (aa 13-195), a transmembrane domain (aa 1,196-1,215), and an intracellular domain (aa 1,216-1,215) (Fig 1a). Similar to other coronaviruses, the SARS-CoV S protein can be cleaved into S1 and S2 subunits by proteases, and a fragment located in the S1 subunit spanning aa 318-510 (192 aa) is the minimal receptor binding domain (RBD) required for binding to the functional viral receptor, human angiotensin-converting enzyme 2 (ACE2). Crystallographic studies reveal that RBD binds to ACE2 through a tyrosine-rich 14 aa receptor binding motif (Figs 1b and 1c). The RBD also contains multiple cysteine residues linked by disulphide bonds, while two residues at positions 479 and 487 of the RBD are associated with enhanced animal to human disease transmission and human to human transmission. SARS-CoV S protein also mediates class 1 viral fusion (1).

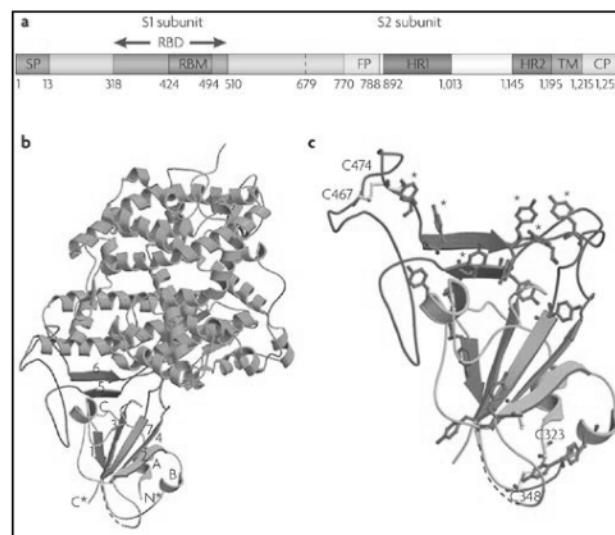
The essential role of S protein in initiating receptor binding and the subsequent fusion between the viral envelop and membrane of susceptible cells makes it not only a major player for determining host specificity, tissue tropism, and viral pathogenicity, but also an attractive target for vaccine and antiviral development. Indeed, S protein-based vaccines developed by different approaches have been proven effective in inducing specific antibodies in many laboratory animal species (e.g., mice, nonhuman primates, etc.) capable of neutralizing homologous SARS-CoV infection in Vero E6 cells and in vaccinated animals. While these full-length S protein-based vaccines are promising candidates, they may induce antibody-dependent enhancement (ADE) of disease in vaccinated animals upon challenge with homologous SARS-CoV (7), thus raising concerns about the safety and ultimate protective efficacy of these vaccines (8).

We have shown that the recombinant RBD (rRBD) antigen of SARS-CoV could be recognized by the neutralizing antibodies in the antisera of mice and rabbits immunized with inactivated SARS-CoV (9). The rRBD also strongly reacts with the convalescent sera obtained from SARS patients, and depletion of the RBD-

specific antibodies from SARS patients results in significant elimination of the neutralizing activity (10). Chen *et al.* (11) have also shown that most neutralizing antibodies of antisera of mice, rabbits and monkeys induced by a live-attenuated MVA virus that expressed the full-length S protein could be absorbed and removed by rRBD. Furthermore, using an RBD fusion protein linked to human IgG1 Fc fragment (designated RBD-Fc) as an immunogen, we have successfully raised high titers ($> 1:10,000$) of neutralizing antibodies in rabbits. Importantly, these antibodies could also effectively cross-neutralize the infection by SARS pseudoviruses bearing S proteins of both homologous and heterologous SARS-CoV isolates, including the representative strains of human 2002–2003 and 2003–2004 SARS-CoV (Tor2 and GD03, respectively) and palm civet SARS-CoV (SZ3). Immunization of mice with RBD-Fc also induced long-term protective immunity against challenge with the homologous SARS-CoV BJ01 strain (12). Administration of an adeno-associated virus (AAV)-based vaccine that contains RBD (RBD-rAAV) by intramuscular and mucosal pathways elicits sufficient neutralizing antibodies to inhibit homologous SARS CoV (GZ50) challenge in the established mouse model, and the immune responses can be enhanced by priming with RBD-rAAV and boosting with RBD-specific peptides. In addition to the B cell-epitopes, SARS-CoV S protein contains T cell-epitopes capable of eliciting CD8+ T-cell responses. One H-2(b)- and one H-2(d)-restricted T-cell epitope were mapped to RBD (S436–S443 and S366–S374, respectively). Immunization of mice with a rRBD-based subunit vaccine (S318–S510) elicits both antibody and cellular immune responses against SARS-CoV (13). Notably, the RBD of S protein contains multiple conformation-dependent epitopes and is the main domain that induces neutralizing antibody and T-cell immune responses against SARS-CoV infection without revealing exacerbated pathogenesis in vaccinated animals in response to viral challenge, making it a desirable target for vaccine development.

To alleviate safety and efficacy concerns inherited by the full-length S protein based vaccines, we will continue and extend our previous efforts and in this proposal fully evaluate the potential of RBD as a subunit of S-protein-based, to be developed as a vaccine. Importantly, the approaches adopted in this proposal for developing an rRBD-based vaccine against SARS-CoV will provide a useful roadmap for designing vaccines against other viruses carrying class I fusion proteins, as these proteins also contain RBDs in their S proteins.

Fig. 1. Schematic representation of the S protein and its RBD (1). (a) Residue numbers of each region represent their positions in the S protein of SARS-CoV. CP, cytoplasm domain; FP, fusion peptide; HR, heptad repeat; RBD, receptor-binding domain; RBM, receptor-binding motif; SP, signal peptide; TM, transmembrane domain. (b) Crystal structures of the RBD complexed with ACE-2. (c) the RBD tyrosine (magenta) and cysteine (yellow) distribution.



Novel Adjuvants. Currently, alum salts are the only adjuvants licensed in the United States (14). To date, two recombinant protein vaccines formulated on alum – hepatitis B virus (HBV) and human papilloma virus (HPV) vaccines – have undergone licensure (14). Their formulation on alum was approved in part on their safety record of alum, together with evidence that alum can stabilize antigens and augment antibody responses to the target antigen (14). However, for both HBV and HPV vaccines the major antigen components also exhibit the ability to self-assemble into virus-like particles, which enhance immunogenicity and elicit high levels of antibody even when alum is used as the solitary adjuvant (15). In contrast, many of the vaccine antigens under development, including RBD, are soluble recombinant proteins. Notably, soluble proteins, such as the malaria antigens AMA-1, MSP-1, and Pfs25, when formulated on alum although had adequate safety profiles in both Phase 1 and 2 clinical trials; they were only modestly immunogenic in terms of the magnitude of antibody titers and the length of time when the antibody titers are detectable (16–19). Similarly, we found that a recombinant hookworm larval antigen when formulated on alum also showed modest antibody titers, although the titers persisted for up to a year after immunization (20). Although in our previous studies, rRBD when formulated in alum induced neutralizing antibodies and protection, it might be necessary to increase the vaccine's efficacy, in particular the induction of cellular immunity, by replacing alum with an innate adjuvant or by adding to the alum formulation a second immunostimulant. One approach is to add a mimetic of a pathogen-associated molecular

pattern (PAMP) that can bind to one or more TLR (21). For instance, TLR9 agonist CpG 7909 oligodeoxynucleotide has been formulated with AMA-1 and other recombinant malaria antigens and shown to augment IgG antibody titers (19,22,23). However, CpG 7909 was also associated with an increase in adverse events including neutropenia, possibly due to immune stimulation and white blood cell sequestration (22-24). In addition, one case of Wegeners granulomatosis was noted to occur in a volunteer who received hepatitis B formulated with an oligodeoxynucleotide similar to CpG 7909 (24,25). An alternative strategy is to use lipid A, the active principle of lipopolysaccharide (LPS), which is a potent TLR4 stimulator (21). To reduce the pyrogenicity and other toxicities, a lipid A from *Salmonella minnesota* has been modified by selective hydrolysis of two moieties (21). The resulting monophosphoryl lipid A (MPL®) is licensed for use in Europe as Fendrix®, a human hepatitis B vaccine (13,26,27). Recently, the biotechnology company, Immune Design Corp. (IDC, <http://immunedesign.com>, Seattle, WA), developed a novel 1.7 kDa synthetic toll-like receptor 4 (TLR4) agonist known as GLA (glycopyranosyl lipid A), which is equivalent of MPL® and is a clinical stage adjuvant being utilized in multiple internal and partnered vaccine programs by our industrial partner, Immune Design Corp. In a study in mice with commercial Fluzone®, a killed influenza vaccine, chemically synthesized GLA was effective in enhancing IgG titers (both IgG 1 and IgG2a), both as an oil-water emulsion (squalene oil and surfactant), and as an aqueous formulation (GLA-AF) (13,26). IL-2 and IFN- γ , Key Th1 cytokines, were also elevated (13). A oil-in-water emulsion of GLA with a malaria vaccine, GMZ2 adjuvanted by aluminum hydroxide, have been also shown to enable the induction of the highest (a) vaccine-specific IgG2a and total IgG titers, (b) parasite-specific IFA titers, (c) levels of IFN- γ , and (d) number of long-lived-plasma cells secreting antibodies, which are thought to be essential for the development of long-term protective immunity against clinical malaria (28). In support of the use of GLA in human trials, in an IND filing this year the FDA has allowed BCM-Sabin to include the GLA as part of the clinical plan of one of our hookworm vaccines, which will initiate phase 1 clinical testing in June, 2011.

(b) Innovation

The use of rRBD, rather than full-length S protein or inactivated or attenuated viruses, for developing SARS vaccines. Our preliminary studies have shown that rRBD is the best candidate for a SARS vaccine since it is highly effective in eliciting high titers of neutralizing antibodies against SARS-CoV and the pseudovirus expressing S protein (29), and it induces protective immunity in animal models against SARS-CoV homologous and heterologous challenge (30). Compared with the inactivated virus and live attenuated virus-based vaccines, the rRBD-based vaccine is much safer since there is no risk to cause virus infection due to the vaccination. It is also safer than the full-length S protein since the linear immunodominant domains in S protein could induce high titers of non-neutralizing antibodies (31), some of which could enhance infection by heterologous SARS-CoV, such as the civet SARS-CoV strains (32). Vaccination of ferrets with vaccinia virus-based SARS vaccine expressing full-length S protein also caused liver damage after animals were challenged with SARS-CoV (7,33). So far, no one has shown that rRBD-based vaccines may induce infection-enhancing antibodies or harmful immune responses (1).

Combining analytical/biochemical tools with biophysical assays to test different excipients and stabilizers and establish an optimal stability profile for the rRBD-based vaccine. In our experience, long-term stability is an essential element for a vaccine that might require emergency stockpiling. Biophysical tools including circular dichroism, intrinsic and extrinsic fluorescence, light scattering, and differential scanning calorimetry have been used previously to evaluate vaccine stability (34,35). Collectively, the combination of these assays during the product development phases will provide specific information concerning the physical state of the protein as a function of stresses (i.e temperature and pH fluctuations). Such data allows the development of specific assays to screen and identify potential stabilizers such as sugars and other buffering agents to prevent degradation. The type of assays (including light scattering for aggregation of recombinant proteins as well as ensuring compatibility of buffering agents with the adjuvants) are determined by the nature of the structural changes of the protein. Once the assays are established, a supplemented GRAS ("generally regarded as safe") library is screened and potential stabilizers identified. The ability of these excipients to prevent aggregation or conformational changes of the protein is a key goal during vaccine formulations.

Preclinical testing of efficacy in two innovative animal models: we will use two stringent and complementary lethal mouse models: 1) MA15/BALB/c mice using a mouse-adapted MA15 virus for homologous virus challenge. It originated from SARS-CoV Urbani strain, which can cause SARS-like disease and mortality in BALB/c mice; 2) GD03/Tg AC70 mice developed by Dr. Tseng at UTMB. Transgenic Tg AC70

mice, express the human angiotensin-converting enzyme 2 (ACE-2), the virus receptor for human SARS-CoV. These mice can be also used for heterologous infections and thus evaluation of cross protection. Following infection of such transgenic mice with SARS-CoV they develop weight loss and other manifestations before reaching 100% mortality within 8 days post-intranasal infection with clinical isolates of SARS-CoV (36). The severity of the disease in Tg AC70 mice makes them valuable for preclinical testing of SARS vaccines (36).

A Product Development Partnership (PDP) to accelerate vaccine product development. An ideal vaccine to prevent SARS would be one that protects against devastating outcomes of the infection after a single dose or two doses spaced closely together so that vulnerable populations could be rapidly immunized in an outbreak setting (see Product Development Strategy Section). In addition, the vaccine should be highly stable so that it can be stockpiled for emergency use. Finally, the vaccine should be high yield and low cost so that it could be used in low- and middle-income country settings in Southeast Asia (where populations are at the greatest risk of acquiring SARS) as well as in the United States. In addition to the innovations described above an added strength of the proposal is using the PDP approach as an innovative first-in-human model for accelerating vaccine product development and entry into clinical trials of the rRBD-based vaccine.

Product Attributes of the rRBD-based Vaccine
1. Effective in a one or two doses
2. Stable for emergency stock-piling
3. Low cost for use in both Southeast Asia and the U.S.

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BCM-Sabin integrates and coordinates industry and academic/public partners and contractors and permits the implementation of innovative business-like practices with traditional basic science approaches and applied product and clinical development technology exchanges. BCM-Sabin has a track record of producing safe and immunogenic recombinant protein vaccines (2). As an added strength, BCM-Sabin is uniquely positioned to develop vaccines at the lowest possible costs so that it can be used in resource-poor settings. This goal has been achieved based on product requirements for its anti-parasitic vaccines intended for use in the poorest countries of Africa, Asia, and the Americas (37). Such economic requirements likely obviate expensive vaccine biotechnologies including mammalian cell culture, insect expression vectors, and prime-boost strategies using adenovirus vectors or DNA vaccines. Therefore, BCM-Sabin focuses its vaccine development strategies on extremely low-cost bacteria and yeast expression vectors (2). BCM-Sabin and its collaborators will employ the PDP approach for innovation which will also include the use of several novel and innovative discovery biotechnologies for adjuvant formulations with IDC's TLR4 GLA adjuvant (13,26,38). In addition, through the application of parallel analytical, biophysical and immunological profiling success will be ensured during process development. A key element of BCM-Sabin's success is regular program/risk management reviews, organized to evaluate each step in the development cycle, by inviting a team of external technical advisors that will include established vaccine development scientists. Such activities will provide the basis for accelerating cGMP manufacture and ultimately a "first-in-human" Phase 1 study of the rRBD-based SARS vaccine.

(c) Approach. The strengths of our approach rely on an extensive evidence base of preliminary data collected over the last seven years by NYBC, which point to the RBD protein as a lead candidate vaccine antigen, together with an eleven-year track record of product development and testing for recombinant vaccines by BCM-Sabin. Additionally, BCM-Sabin has had previous success in tech transferring processes for recombinant protein vaccines to WRAIR our cGMP contractor (CMO) of choice. Further, our group has demonstrated the feasibility of developing rRBD-based subunit vaccines. We have shown that mammalian cell-expressed rRBD fused induces high titer of RBD-specific neutralizing antibodies in vaccinated animals and long-term (over a year) immune responses and protection against subsequent SARS-CoV challenge (12), while using the pseudotyped viruses expressing S protein originated from Tor2, GD03, or SZ3, the representative strains of human 2002–2003 & 2003–2004 SARS-CoV and palm civet SARS-CoV, respectively, we also found that mouse and rabbit antibodies raised against rRBD derived from either one of the aforementioned strains of SARS-CoV and the palm civet SARS-CoV cross-neutralize one another, suggesting their protective efficacy against challenge with heterogeneous viruses (39). We have demonstrated that immunization of mice with rRBD derived from various expressing systems, including mammalian cells (293T and CHO), insect sf9 cells, and *E. coli* was capable of producing high-levels of RBD-specific neutralizing antibody and potent T cell responses against both pseudotyped and live SARS-CoV. Importantly, these rRBD proteins appear to maintain intact conformation and authentic antigenicity, reacting with the RBD-specific and conformation-dependent mAbs with neutralizing activity (30,40,41). These rRBD proteins elicited immunity that protected all vaccinated mice from SARS-CoV challenge (30). These preliminary data and expertise by our collaborators at NYBC, will now be paired with the track record at BCM-Sabin/WRAIR for transitioning recombinant protein-based

vaccines through process development and formulation, technology transfer for cGMP manufacture, lot release and stability testing, and regulatory filing with the FDA.

Overview: In response to this RFA, BCM-Sabin will apply its proven product development partnership approach and strong peer-reviewed publication track record in research and development of parasitic recombinant protein vaccines to accelerate the development of a recombinant RBD-based vaccine to prevent SARS-CoV infection. To ensure success, BCM-Sabin will provide program management throughout the project timeline and leverage the collaborations with the research and industry partners to augment the expertise for anti-SARS-CoV vaccine testing. A strength of the proposal are based on the supporting proof of concept data that identifies the RBD protein as a lead candidate vaccine antigen selected for further process development, characterization and preclinical evaluation. Following feasibility of expression in both bacterial and yeast expression systems, the recombinant proteins will be assessed at BCM-Sabin for scalability, yield, quality and stability. Antigenicity, functionality, immunogenicity and potency of the vaccine formulations will also be developed and evaluated at NYBC and UTMB. Furthermore, rRBD protein from the selected expression system will be compared at NYBC and UTMB for its ability to induce neutralizing antibodies and protection in laboratory animals against challenge infections with multiple strains of the SARS-CoV using different adjuvant platforms; alum (either Alhydrogel® or aluminum phosphate) and/or GLA, a TLR4 antagonist adjuvant. Once the most efficacious expression system is selected, BCM-Sabin will perform scale-up process development (PD) at the 10 liter fermentation scale followed by protein purification under detailed documentation. Reproducibility will be confirmed and specific product quality control assays will be developed in collaboration with NYBC and used to characterize rRBD. In addition, vaccine buffer formulations will be developed and characterized, and excipients and stabilizers will be evaluated. The stabilized molecule will then be formulated with alum and/or GLA and their effect on the structure stability will also be examined. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing.

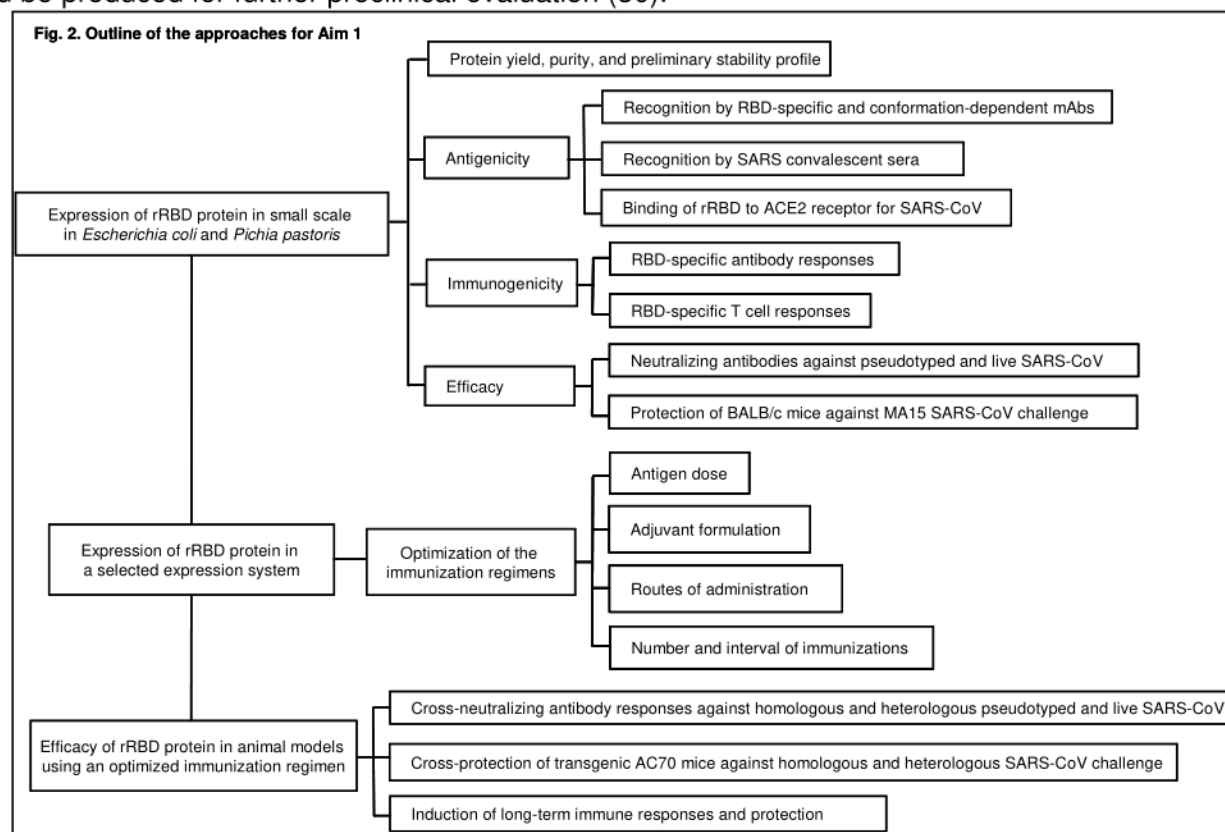
The Major Deliverables

1. Development of a scalable, reproducible process for expression of the rRBD protein and formulation of the RBD-based vaccine with high quality and stability.
2. Establishment of an immunization regimen, including the optimized antigen dose, vaccination schedule and route and adjuvant formulation, for evaluation of the immunogenicity and efficacy of the vaccine.
3. Successful pilot cGMP manufacture of a subunit SARS vaccine comprised of rRBD protein and an adjuvant formulation.
4. Completion of the GLP toxicology testing with acceptable safety profile.
5. IND preparation and submission with U.S. FDA.

The developed process will then be transferred to WRAIR that successfully manufactured two hookworm vaccines with BCM-Sabin. After pilot lot material is produced and released and following a pre-IND meeting with the U.S. FDA, toxicology testing will commence and an IND application will be prepared. Thus a key strength is clear-cut project deliverables (see adjoining box).

Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. Two protein expression systems, *E. coli* and *Pichia pastoris*, will be evaluated in parallel for the feasibility of scalable expression of the rRBD protein. Preliminary studies have shown that *E. coli*-expressed rRBD protein was able to elicit high levels of neutralizing antibodies and protection in an animal model (30). However, the bacterially expressed protein was not evaluated and formally characterized for suitable scalable vaccine processes hence a more formal evaluation is proposed. While we have no knowledge whether the yeast expression system will be better than a bacterial system, BCM-Sabin has strong capabilities for both systems. To date at least two hookworm vaccine antigens, Na-ASP-2 and Na-GST-1, expressed by BCM-Sabin were shown to be suitable for producing high resolution crystals for X-ray diffraction (42,43), and were highly protective in animal models - these vaccines have already advanced through product and clinical development pathways (2,20). An expression systems will be selected for the subsequent studies based on the following criteria: 1) the yield, scalability, purity and preliminary stability profile of the rRBD protein expressed; 2) the antigenicity - recognition of rRBD by conformation-dependent monoclonal antibodies (mAbs) and by human convalescent sera, and the functionality – ability of rRBD to bind to ACE2 receptor for SARS-CoV; 3) the immunogenicity - the ability of the rRBD to elicit RBD-specific humoral and cellular immune responses; and 4) the efficacy – the ability of the rRBD to induce neutralizing antibodies and protection in vaccinated mice against subsequent lethal challenge with SARS-CoV Urbani strain (MA15/BALB/c mouse model). Once the most optimal expression system is selected, it will be used to generate research lots of rRBD protein for further characterization and confirmatory studies as well as for optimization of immunization

regimens by testing different (i) antigen doses and adjuvant formulations, and (ii) routes of administration, number and interval of immunizations. The efficacy of the rRBD-based SARS vaccine to induce neutralizing antibodies, protection and long-term immune responses will be evaluated in a SARS-CoV infection mouse model (GDO3/Tg AC70) using the optimized immunization regimen (Fig. 2). **The strength of this aim is two-fold:** 1) BCM-Sabin already has ample expertise in the evaluation of expression systems to determine the suitability for a molecule entering into a vaccine development pathway; and 2) preliminary data with rRBD expressed using a bacterial expression system at the shake-flask has been shown to elicit high levels of neutralizing antibodies and protection in an animal model, which provides proof of principle that this molecule could be produced for further preclinical evaluation (30).



1.A. Feasibility of scalable expression. BCM-Sabin has extensive experience with bacterial and yeast expression systems and in efforts at both the shaker-flask and up to 10 L fermentation level to optimize scalable expression. As evidence of our track-record in protein expression, four recombinant antigens derived from either hookworm or schistosomes have been successfully expressed at a level of purity and yield ideal for the creation of crystals and solving crystal structures as well as for use in vaccine trials in humans (42-45).

1.A.1. *E. coli* and yeast expression systems. We will use a parallel approach to evaluate both expression systems. Briefly, for bacterial expression, the NYBC investigators previously evaluated the pET-SUMO *E. coli* system (Invitrogen) and pET14b for the expression of rRBD protein, thus providing proof of principle that a bacterial system may be suitable for this protein. The highly-purified rRBD expressed from these systems reacted with the conformational epitope-specific mAbs targeting S protein, suggesting that this rRBD protein maintain intact conformation and authentic antigenicity. These proteins were able to induce strong neutralizing antibody responses and full protection in the vaccinated mice (30). Moreover, the pET-SUMO expressed rRBD was able to induce memory when formulated with alum. A strength of this sub-aim is BCM-Sabin's history and expertise in scaling up recombinant protein antigens both in *E. coli* and in yeast. Because we have found that some proteins expressed with SUMO tags may not be suitable for scale-up and downstream manufacture, we propose to evaluate additional and possibly more suitable *E. coli* systems. Some of these systems include the use of traditional pET/BL21 systems, as well pTrcHis/BL21, and pBAD (araBAD promoter), and the cloning of either harmonized or optimized RBD sequences. Since yeast expression systems have proven to overcome production hurdles of the *E. coli* system, the rRBD DNA sequence will also be optimized and engineered into several yeast expression vectors such as pPICZαA and pPink and evaluated for antigenicity and

immunogenicity. Initial cell growth and protein expression will be conducted in shake flasks and protein will be purified by column chromatography.

1.A.2. Protein yield, purity, and preliminary stability profile. The yield and purity of rRBD will be determined by assays such as HPLC (RP-HPLC and/or SE-HPLC), protein concentration, SDS-PAGE stained with Coomassie Brilliant Blue or Silver Stain, Mass Spectrometry and Residual Host Proteins (by slot-blot) as previously described (30,46). The preliminary stability profile of rRBD protein will also be determined using the assays above and by others such as circular dichroism, intrinsic and extrinsic fluorescence and light scattering (34,35). Collectively, the combination of assays during the development phases provides specific information concerning the physical state of the protein as a function of stresses (i.e temperature and pH fluctuations).

1.B. Antigenicity and functionality. For this sub-Aim we will build on a 7 yr track record of evaluating SARS vaccine candidates in preclinical testing leading to the identification of rRBD as a key protective antigen.

1.B.1. Antigenicity of rRBD protein. The antigenicity of the rRBD protein expressed in the different expression systems will be evaluated by a sandwich ELISA using RBD-specific and conformation-dependent mAbs, including 33G4 (Conf V), 24H8 (Conf I), 31H12 (Conf II), and 35B5 (Conf IV) (40), and human convalescent sera from at least three different SARS patients containing neutralizing antibodies (47). Briefly, rRBD protein at 1 µg/ml will be coated onto the plate, followed by addition of the mAb at 10 µg/ml or the convalescent sera at 1:100 dilution. After addition of HRP-labeled anti-mouse or anti-human IgG, respectively, the binding of antibodies to the coated rRBD protein will be measured. Recognition of the rRBD by these mAbs will also be assessed by Western blot using native and SDS-PAGE gels (30).

1.B.2. Functionality of rRBD protein. The RBD in the SARS-CoV S protein is responsible for virus binding to host cell receptor – ACE2 and mediates the viral entry into the host cell (1,48,49). Our previous studies have shown that the recombinant RBD and S1 proteins could bind to the soluble and cell-associated ACE2, and that anti-RBD antibodies could significantly inhibit the binding of rRBD and rS1 to ACE2 molecule (29,50,51). We will evaluate the binding activity of the differently expressed rRBD proteins to ACE2 by ELISA. Briefly, recombinant soluble ACE2 (R&D Systems, Minneapolis, MN) at 2 µg/ml will be coated onto a plate, followed by addition of rRBD, HRP-conjugated anti-RBD antibody, and the substrate, sequentially. The OD value will be measured (29,50,51). The binding of rRBD to sACE2 can also be detected by isothermal titration calorimetry (ITC) (52,53). The binding of rRBD to the cell-associated ACE2 will be measured by flow cytometry (29,50,51).

1.C. Immunogenicity. The immunogenicity of the rRBD protein will be evaluated by assessing RBD-specific antibody and T cell immune responses in mice immunized with rRBD plus alum as the adjuvant using our previously optimized vaccination regimen (priming subcutaneously with 20 µg and boosting twice with 10 µg rRBD three-weeks apart)(30).

1.C.1. Anti-RBD-specific antibody responses. The sera of the rRBD-immunized mice will be collected 10 days after each boost and the anti-RBD antibody titers will be determined using ELISA as described above (1.B) (30). To measure the isotypes of the RBD-specific antibodies in mouse sera, the HRP-conjugated goat anti-mouse IgG will be replaced by HRP-conjugated goat anti-mouse IgG1, IgG2a, IgG2b, IgG3 and IgA (54).

1.C.2. RBD-specific T cell responses. The RBD-specific T cell responses will be assessed using spleen and lymph node cells collected at day 10th after the last immunization by analyzing MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte (CTL) responses using ELISPOT (Mabtech, Mariemont, OH) following *in vitro* priming with peptides encoding specific CD8 or CD4 epitopes previously identified by us (55,56). Briefly, 96-well ELISPOT plates will be coated with anti-IFN-γ, -IL-2, -IL-4 and -IL-10 mAbs. Single-cell suspensions prepared from the spleens and lymph nodes of the vaccinated mice will be added to the coated plate (2x10⁵ cells/well), followed by an incubation at 37 °C for 24 h in the presence of MHC-H-2d restricted SARS-CoV-specific cytotoxic T lymphocyte (CTL) peptide (N50) or Th peptide (N60), and then with biotinylated labeled anti-mouse IFN-γ, IL-2, IL-4 and IL-10 mAbs. Spots of cytokine-producing T cells will be counted and expressed as the number of spot-forming cells (SFC) per 10⁶ input cells. If necessary, a more complete cytokine profile will be obtained by evaluating culture supernatants three days after *ex-vivo* stimulation using Bioplex/Milliplex methodology (57).

1.D. Efficacy. We previously demonstrated that rRBD-immunized aged mice with serum neutralizing antibody titers ranging from 1:189 to 1:505 were fully protected from SARS-CoV challenge (12). Other groups reported that mice immunized with attenuated virus or DNA vaccines encoding the SARS-CoV S protein, or inactivated virus had serum neutralizing antibody titers ranging from 1:50 to 1:640 and most of these mice were protected from SARS-CoV challenge (32,58,59), suggesting that the level of neutralizing antibody response is correlated

with the efficacy of protection and that a vaccine that induces in the immunized mice neutralizing serum antibody titers of 1: >500 would be considered as highly effective. Therefore, the efficacy of the rRBD expressed in the different expression systems will be judged based on their abilities to induce both neutralizing and protective responses when formulated in alum (30,40,55,57).

1.D.1. Neutralizing antibody responses. The titers of the neutralizing antibodies in the sera of mice immunized with the rRBD proteins will be determined using the pseudotyped and live SARS-CoV by Dr. Jiang's group at NYBC and Dr. Tseng's team at UTMB, respectively. The titer of serum neutralizing antibodies against SARS pseudovirus will be determined as previously described (29,30). Briefly, the pseudotyped viruses expressing SARS-CoV Tor2 S protein will be preincubated with serially diluted mouse sera at 37 °C for 1 h before adding to the ACE-2 expressing cells. Fresh medium will be added 24 h later, followed by lysis and the addition of the luciferase substrate (Promega). Relative luciferase activity will be determined in Ultra 384 luminometer. The 50% neutralization titer (NT₅₀) will be calculated using CalcuSyn program (60). Positive control will be neutralizing Mab's and negative control will be pre-immune mice sera. The neutralization assay against infection by live SARS-CoV Urbani strain will be performed as previously described (30,40,55). In brief, serial 2-fold dilutions of sera will be mixed with 100 TCID₅₀ of SARS-CoV, incubated at 37 °C and added to the monolayer of Vero E6 cells in triplicate. Cytopathic effect (CPE) in each well will be recorded on day 3 post-infection. The neutralizing titers that completely prevent CPE in 50% of the wells (NT₅₀) will be calculated (61).

1.D.2. Protection against SARS-CoV challenge. The mouse models have been widely used for evaluation of the *in vivo* efficacy of SARS vaccines and passive transfer of protective antibodies (32,58) because mice are much less expensive and more convenient for handling than ferrets and monkeys. It was reported that a mouse-adapted MA15 virus originated from SARS-CoV Urbani strain could cause SARS-like disease and mortality in BALB/c mice (62). Thus, we will use this well-established mouse model for evaluating the efficacy of rRBD to induce protection in the immunized animals. Ten days after the last boost, mice will be lightly anesthetized and bled for determining the titers of neutralizing antibodies prior to intranasal (i.n.) inoculation with 10⁵ TCID₅₀ of MA15 virus in 50 µl. For each vaccinated and non-vaccinated group (n=15 per group), five mice will be sacrificed 2 days post-challenge for collection of lung tissues for detecting viral titers by using Vero E6 cells as described above (1.D.1) and expressed as TCID₅₀/g of lung tissue, and for examination of histopathogenic changes. In addition, Bioplex analyses will be performed for profiling cytokine and chemokine responses of the infected lungs. The remaining 10 mice in each group will be monitored for their weight losses, clinical symptoms and mortality rate for at least 14 days, as previously described (63). Because antibody-dependent enhancement (ADE) is a genuine concern of the overall vaccination strategy against SARS-CoV (64,65), to ascertain that rRBD protein is an effective and safe vaccine candidate, we will also investigate whether the rRBD could induce eosinophilia and other harmful inflammatory responses in the infected lungs.

Anticipated outcomes. Based on the assays described above, each expression system will be scored and ranked on the basis of a number of parameters as outlined in Table 1. This selection system is a key strength and we have determined previously that ranking by this mechanism helps to identify the most promising expression systems for vaccine candidates for subsequent process development and manufacture. An rRBD antigen preparation with a category score < 3 or final score < 15 will be removed from further consideration. A lead rRBD from one of the expression vectors with the highest final score will be selected for subsequent studies for optimization of immunization regimens (Aims 1E-G), and process development and pilot cGMP manufacture (Aims 2 and 3).

1.E. Optimization of the immunization regimens. Induction of high levels of neutralizing antibody and protection responses is dependent on not only the antigenicity and immunogenicity of the antigen, but also the optimal antigen dose, adjuvant formulation, administration route, and number and interval of immunizations. Through our partnership with IDC, we have access to a novel adjuvant, GLA that can be formulated on its own or in the presence of alum. Thus, we will also optimize vaccine formulations using the rRBD protein from the selected expression system. The goal is to find the best vaccine formulation and regimen that induces highly potent neutralizing antibody and CTL responses as well as protection against homologous and heterologous virus challenge, while using the least amount of rRBD antigen within the vaccine and number of immunizations.

1.E.1. Optimization of antigen dose and adjuvant formulation. In our preliminary studies, s.c. vaccination of 20 µg followed by two 10 µg boosts of *E. coli* expressed rRBD plus Sigma Adjuvant System (SAS) or Alum was able to induce effectively strong neutralizing antibody and protective immunity (30). For optimizing both dosing and adjuvant formulation, we will immunize (s.c.) BALB/c mice with three different concentrations of

rRBD (e.g., 5, 10, or 20 µg) in the presence of alum (Alhydrog el® or aluminum phosphate) and/or GLA and boost twice at 3-week intervals. The immunogenicity of the differentially formulated rRBD vaccines will be assessed and compared, according to established protocols as described above, in order to select the most effective dose and formulation of the rRBD-based vaccines for the subsequent studies.

1.E.2. Optimization of route of administration, number and interval of immunizations. An appropriate route, and number and interval of immunizations are important for determining the nature (e.g., quantitative and quality) of effective immune responses. To optimize these, BALB/c mice will be immunized subcutaneously (s.c.), intramuscularly (i.m.) or intradermally (i.d.) with rRBD protein with the optimized dose-and-adjuvanted rRBD, as selected above in 1E1. The animals will be either boosted once at 2- or 4-week intervals or not boosted. Mouse sera, spleens and lymph nodes will be collected 10 days after last boost for detection of the RBD-specific antibody and T cell immune responses as described above (1.C).

Table 1. Proposed antigen ranking system

1. Expression Feasibility (yield & purity)	2. Antigenicity and functionality	3. Immunogenicity	4. Induction of neutralizing antibody responses	5. Protection against SARS-CoV challenge	Final Score (tally of scores from each category)
0-5	0-5	0-5	0-5	0-5	0-25
rRBD expression feasibility: A major impediment to the success of efficacious vaccine antigens is the ease and cost-effectiveness with which their production can be scaled-up to cGMP manufacture. 0 = unfeasible; 1 = low yields but potentially feasible; 2 = modest yields but unstable protein 3 = modest yields and relatively stable protein but significant process development required; 4 = modest yields and relatively stable protein but feasible through process development approaches; 5 = high yield of soluble, stable protein with straightforward scale-up for manufacture. Antigenicity and functionality of rRBD: (1) Recognition by conformation-specific mAbs targeting RBD will be determined by an ELISA using rRBD protein (1 µg/ml) as the coating antigen and the mAb 38D4 at 10 µg/ml (40). The score will be recorded based on the OD value: 0 = <0.1; 1 = 0.1 – 0.25; 2 = 0.25 - 0.49; 3 = 0.5 – 0.74; 4 = 0.75 – 1.0; and 5 = >1.0; (2) Recognition by convalescent sera will be measured by an ELISA with rRBD protein (1 µg/ml) as the coating antigen and the SARS convalescent sera at 1:100 dilution (47) and scored based on the OD value: 0 = <0.1; 1 = 0.1 – 0.25; 2 = 0.25 - 0.49; 3 = 0.5 – 0.74; 4 = 0.75 – 1.0; and 5 = >1.0; (3) functionality of rRBD protein. The ability of rRBD to bind ACE2 receptor for SARS-CoV will be measured by ELISA using rACE2 as the coating antigen (2 µg/ml) and rRBD protein at 1 µg/ml (51), and scored based on the OD value: 0 = <0.1; 1 = 0.1 – 0.25; 2 = 0.25 - 0.49; 3 = 0.5 – 0.74; 4 = 0.75 – 1.0; and 5 = >1.0. The scores of (1), (2) and (3) will be averaged and entered into category 2. Immunogenicity of rRBD: (1) RBD-specific antibody responses will be assessed with an ELISA with rRBD protein as the coating antigen (40) and scored based on the serum titers anti-RBD antibodies (reciprocal dilution): 0 = < 10²; 1 = ≥ 10² ~ < 10³; 3 = ≥ 10³ ~ < 10⁴; 4 = ≥ 10⁴ ~ < 10⁵; and 5 = ≥ 10⁵. (2) RBD-specific CTL responses will be tested with an ELISPOT (55) and scored based on the number of IFN-γ+ CD8+ cells in 10⁶ splenocytes: 0 < 5; 1 = ≥ 5 ~ < 15; 2 = ≥ 15 ~ < 25; 3 = ≥ 25 ~ < 35; 4 = ≥ 35 ~ < 45; and 5 = ≥ 45. The scores of (1) and (2) will be averaged and entered into the category 3. Induction of neutralizing antibody responses: Neutralization of pseudotyped virus bearing SARS-CoV S protein (1) and live SARS-CoV (2) will be determined as we previously described (40,55) and scored based on the serum titer of 50% neutralization (NT₅₀): 0 = < 20; 1 = ≥ 20 ~ < 100; 2 = ≥ 100 ~ < 250; 3 = ≥ 250 ~ < 500; 4 = ≥ 500 ~ < 1,000; and 5 = ≥ 1,000. The scores of (1) and (2) will be averaged and entered into category 3. Protection against SARS-CoV challenge: Protection of BALB/c mice against MA15 SARS-CoV challenge will be determined as described (19, 24). The score will be recorded based on: (1) viral burden within the lungs (TCID₅₀/g of lung tissue): 0 = 5 out of 5 (5/5) or 100% detectable; 1 = 4/5 or 80% detectable; 2 = 3/5 or 60% detectable; 3 = 2/5 or 40% detectable; 4 = 1/5 or 20% detectable; and 5 = 0/5 or 0% detectable. (2) Body weight lose: 0 = > 20%; 1 = 15-20%; 2 = 10-15%; 3 = 5-10%; 4 = 0-5%; and 5 = ≤ 0%. (3) Accumulated mortality rate (%): 0 = 80-100%; 1 = 60-80%; 2 = 40-60%; 3 = 20-40%; 4 = 10-20%; and 5 = 0%, and (4) Protective efficacy against histopathological changes: 0 = severe, > 50% area; 1 = severe, < 50% area; 2 = moderate; 3 = mild; 4 = minimal/nonspecific; and 5 = none or negligible. The scores of (1), (2), (3), and (4) will be averaged and entered into category 5.					

1.F. Efficacy of rRBD-based vaccine to induce cross-neutralizing antibody responses and cross-protection in mice vaccinated with rRBD using the optimized immunization regimen. Based on the above studies, we will select an optimized immunization regimen for vaccination of mice with the most effective adjuvanted rRBD vaccine and evaluate the cross-neutralizing antibody responses and cross-protection against infection by homologous and heterologous SARS-CoV strains

1.F.1. Cross-neutralizing antibody responses. We will immunize BALB/c mice with the most effective adjuvanted rRBD vaccine using an optimized immunization regimen. The titers of neutralizing antibodies (NT₅₀) will be determined in sera collected 10 days after the last boost as described above (1.D.1) (29,30) against the pseudotyped viruses expressing the S protein of homologous and heterologous SARS-CoV strains Tor2, GD03, and SZ3, the representative strains of human 2002–2003 and 2003–2004 SARS-CoV and palm civet

SARS-CoV, respectively (39). The antisera will also be tested against the live homologous SARS-CoV Urbani strain (its S protein sequence is 100% identical to Tor2) and heterologous SARS-CoV strain GD03.

1.F.2. Cross-protection. While infection of BALB/c mice with MA15 virus is a useful lethal model for assessing conveniently the vaccine efficacy, this animal model cannot be used for evaluating the efficacy of anti-SARS vaccines to induce cross-protection against heterologous SARS-CoV strains. To evaluate such efficacy the transgenic mouse model developed by Dr. Tseng's group at UTMB will be used. Briefly, four groups of transgenic AC70 mice (N=15 each group) will be immunized with the adjuvanted rRBD protein using the optimized vaccination regimen or remain non-vaccinated, as control. Ten days after the last immunization, mice will be bled for assessing PRNT₅₀ prior to challenging with 10³ TCID₅₀ (~ 10X LD₅₀) live homologous SARS-CoV strain Urbani and heterologous SARS-CoV strain icGD03-S, which bears the S protein gene of the most genetically divergent human strain, GDO3 (66), and which could efficiently replicate in mouse lungs and in human airway epithelial cells (64). Ten of the challenged mice in each group will be monitored for their weight losses, clinical symptoms and mortality rate for at least 14 days, and the remaining five of challenged mice in each group will be sacrificed 2 days post-infection for collection of lung and brain tissues for detecting viral titer and for examining histopathogenic changes (63) as described above (1.D.2).

1G. Efficacy of the rRBD-based vaccine to induce long-term immune responses and protection. Our preliminary studies show that immunization of rRBD with Freund's adjuvant induced long-term (as up to one year) and potent SARS-CoV S-specific antibodies with strong neutralizing activity, and protected mice against subsequent SARS-CoV challenge (12). We will fully assess the potential of the rRBD-based vaccine in providing long-term immune responses and protection against MA15 SARS-CoV infection in BALB/c mice. Briefly, we will immunize a total of six groups of BALB/c mice (N=20 per group) using the optimized vaccination regimen; three with adjuvant only as control groups, the other three groups with adjuvanted rRBD as test groups, thereby resulting in three pairs of "control and test" groups of animals. Five mice in each paired group will be sacrificed 10 days, 6 months and 12 months, respectively, after the last boost for collection of sera, spleen and lymph nodes for evaluating RBD-specific antibody responses, including neutralizing antibodies, and T cell responses as described above (1.C. and 1.D.1) (29,30). The remaining 15 mice in each paired group will be challenged with 10³ TCID₅₀ SARS-CoV Urbani strain 10 days, 6 months and 12 months, respectively, after the last boost. Five challenged mice in each paired group will be sacrificed at day 2nd after infection for assessing viral yields and histopathological changes in the lungs, whereas the remaining 10 mice will be monitored daily for the onset of clinical manifestations (e.g., weight losses) and accumulated mortality, as described above (1.D.2) (63).

Additional anticipated outcomes. In summary, we will use a series of well-optimized methodologies and two novel and complementary mouse models that have been developed in our laboratories to fully evaluate the vaccine potential of rRBD protein against SARS-CoV-induced illness and mortality. It will be based on five highly stringent criteria, including (i) expression feasibility, (ii) antigenicity and functionality, (iii) immunogenicity, (iv) ability to elicit neutralizing antibodies, and (v) capacity to induce protection against SARS-CoV challenge. We anticipate establishing a reproducible expression system suitable for the scale-up production of the proven effective candidate vaccine. Although all the five criteria will be closely followed for the selection (each of the category score must be ≥ 3), we will put more weight on the protective efficacy in vaccinated animals since the major outcome endpoints are titers of the neutralizing antibodies and levels protection of vaccinated animals against SARS-CoV infection. Therefore, we will select the antigen with the scores of categories (iv) and (v) at 5. To avoid an undesirable bias, each experiment will be repeated twice and the positive and negative controls will always be included.

Specific Aim 2: Process development, characterization, formulation and stability profiling. We will extend the initial work at the shake flask level described in Aim 1 to generate a research cell bank and optimize expression of rRBD in the selected expression system. To improve yield, purity, stability and activity of the soluble protein, the optimal growth and induction conditions for the fermentation processes will be determined. Temperature, pH, dissolved oxygen, and agitation will be evaluated for their effect on cell growth, protein expression and activity. Once the best parameters are selected, these will be evaluated up to the 10L scale, with the aim of reproducible high-yield production (minimum of 100 mg/L target). The major sub-aims include: (A) production and purification of the rRBD protein at the 10 liter fermentation scale and using ion-exchange, hydrophobic interaction, and other column chromatographies. (B) Development of product specific assays for use to follow the expression and purification in process and for purposes of downstream lot release of clinical lots. (C) Documentation of three successive process development runs comprised of both fermentation and protein purification. (D) Product stability studies to establish a profile of the clinical grade lots.

2.A. Development and optimization of a 10L scale process (fermentation & purification). For fermentation protein expression bioreactors (BioFlo systems from New Brunswick Scientific) will be used. Scale up experiments will be performed with the selected expression system to determine the optimal induction conditions for the fermentation processes. This will be assessed by first optimizing a series of small scale fermentations. Once the best parameters are selected these fermentation parameters will be evaluated up to the 10 L scale, with the aim of reproducible high-yield production. Briefly, the fermentation will be optimized by evaluating six basic areas: growth rate, temperature, pH, dissolved oxygen, shear sensitivity, and foam. As for fermentation, purification processes will be started at the small scale, and these also are developed with the aim of keeping costs as low as possible. The AKTA Explorer and Pilot purifier systems and software will be used. Each isolation procedure will be carefully analyzed and assessed for its ability to improve both protein yield and purity. Both cation and anionic ion-exchange resins will be used as well as hydrophobic affinity resins, and gel filtration resins when necessary. For the overall process development strategy, purification is typically conducted at the 1/6 scale, meaning that if the desired cGMP manufacturing run will be conducted using a 60 L fermentation, purification would be developed using 10 L fermentations that represent a one-sixth scale purification. Alternative strategies used before (35) will be tested to increase yield, purity, and stability.

2.B. Assay development. Protein-specific assays (described further in the product development strategy) will be developed to characterize the rRBD during development (in-process), post-production and post-formulation. The same assays will establish the chemical stability. This aim will ultimately allow for future qualification of the assays to confirm accuracy, precision, sensitivity, specificity, reproducibility, and robustness. Based on these qualification procedures, a set of release specifications are established to support the GMP manufacturing and downstream regulatory package of the recombinant antigen vaccine. The majority of these assays are antigen-specific, and will be developed through application and/or modification of methods currently existing in our laboratories, including HPLC, SDS-PAGE (under reduced and non-reduced conditions), N-Terminal sequencing, Mass Spectrometry and ELISA, among others. These assays establish identity, yields, purity, conformation and integrity after expression. All assays will be complemented with biophysical methods such as light-scattering. Careful attention will be placed on the evaluation of endotoxin levels and the analysis of residual host cell protein (HCP) impurities. The HCP method, includes the use of a slot blot system with scanning laser densitometry to allow picogram level sensitivity in the detection of residual HCP in the in process fermentation products and final purified protein samples. Briefly, proteins will be analyzed using a generic rat polyclonal pool of sera with antibodies that are generated against soluble and insoluble proteins. Furthermore, analysis of contamination by exogenous endotoxin with Limulus amoebocyte lysate will be done.

2.C. Execution of 3 successive process development runs at the 10L scale. Upon the completion of the optimization of process development and assay development, we will demonstrate reproducibility with three consecutive production and purification runs at the 10L scale. Such reproducibility is a key strength of our approach to process development. The consecutive batches will be characterized by using the in-process and release quality control testing. This activity is critical for the future success during technology transfer to a manufacturing facility. Based on the experiences of BCM-Sabin all reagents, processes and equipment used at the 10L scale should be compatible to those to be used during manufacture. At the time of transfer, scale will be the only variable that will be modified to avoid run failure.

2.D. Formulation and Stabilization. An important strength is the pairing of GRAS-designated excipients with biophysical techniques in order to produce a formulation of a recombinant vaccine of maximal stability potentially suitable for emergency stockpiling. Pre-formulation studies of rRBD in solution will provide key information for selecting appropriate buffer systems, optimal pH and stabilizers for a potent adjuvanted vaccine. Briefly, characterization with biophysical techniques including light scattering, circular dichroism, and intrinsic and extrinsic fluorescence will provide information concerning the physical state of the vaccine protein as a function of temperature and pH (34,35). GRAS-designated excipients and stabilizers such as sugars and other buffering agents will be evaluated to maximize the stability of the rRBD in solution. Temperature, pH and oxidizing agents will be used to induce deamidation and oxidative stress, which will be assessed with isoelectric focusing studies. In addition, accelerated stability protocols will be developed and assessed at different time points and after doing temperature excursion experiments (repeated freeze thaw cycles, and different temperatures). The stabilized molecule will then be formulated with alum (Alhydrogel® or aluminum phosphate), GLA or in combination, based on the results contained in Aim 1E. There is no way to predict whether Alhydrogel® vs. aluminum phosphate is the preferred form of alum. Assays to establish the percentage of protein bound with different combinations of the aluminum salts will be developed to ensure that

buffers and excipients present in the formulation do not interfere with binding capacity. Titrations of both antigen and adjuvant will be conducted. After the generation of binding isotherms, desorption studies will be performed using increasing amounts of sodium chloride, citrate buffer and a mild chaotrope. Desorption of antigens with increasing salt concentration is highly suggestive of electrostatic binding of the protein to adjuvant. Alternatively, citrate can sometimes be used to dissolve aluminum salts. In addition, formulations will be established both biochemically and by immunological potency. The interaction between rRBD with alum and/or GLA and its effect on stability of the protein will be examined based on IDC recommendations.

Anticipated outcomes/Alternative approaches: It is anticipated that by following well documented production processes, these experiments will result in a very consistent, high yield fermentation and purification process at the 10L scale. Deficits in any given process development run would be approached by investigating and establishing corrective actions and embarking on additional runs. If necessary, temperature, pH, dissolved oxygen, agitation and other parameters will be further evaluated performing Design of Experiments (DoE) to establish robustness of the different steps of fermentation and purification (35). DOE is an important strength for advancing candidate antigens through process development. However, based on the prior success of the small scale expression (1L) from Aim 1, we envision that the process will be easily scaled up directly from 1L to 10L scale of production. We expect a low probability that the 10L process scale up may lead to difficulties since the proposed activities will be maximized from the information obtained from Aim 1 that will provide feedback for how to improve the in-process procedures. We try to avoid the use of polyhistidine and other peptide tags as well as nickel affinity resins. However, in the event of low yield or inefficient purification processes using ion-exchange, hydrophobic interaction, and gel filtration, this approach remains a possibility. The buffer formulation and storage conditions of the purified protein will be optimized to support stability with and without adjuvant.

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. The process development and formulation technology for the selected rRBD-based vaccine will be transferred to the WRAIR pilot cGMP facility, an experienced vaccine manufacturer with a track record of working with BCM-Sabin for successful tech transfer (20,67). Assays developed and qualified in Aims 1 and 2 will then be used for lot release and to initiate stability testing. The protein-specific assays will be conducted at BCM-Sabin, the antigenicity/immunogenicity and efficacy will be conducted at the NYBC, while other regulated assays will be contracted to industrial partners. BCM-Sabin will be providing regulatory, quality assurance and link our project with the industrial partners for: 1) cGMP manufacture; 2) access to GLA, a TLR4 adjuvant from Immune Design Corp. and 3) GLP toxicology testing. Following a pre-IND meeting with the FDA GLP toxicology testing at Frontier Biosciences will be performed and an IND prepared.

3.A. Strategy for Manufacture of Drug Substance and Drug Product. Based on our previous success with technology transfer CMOs (contract manufacturing organizations) in the U.S. and Brazil, we expect that the production processes will yield rRBD-based vaccine of sufficient yield, purity, and stability for use in Phase 1 clinical testing. An investigational biologic must be manufactured in a prescribed manner, within a controlled environment, and by experienced staff. Initially the technology transfer staff will provide the research clone for the generation of Master and Production Cell Banks. Following fermentation and purification, antigens then undergo final formulation, which will involve the addition of stabilizers or diluents. Steps are taken to ensure final purity, sterility, and potency. The active ingredient (rRBD) is the “drug substance” formulated with buffers, excipients and adjuvants. Final fill is the formulated and vialled vaccine, the “drug product.”

3.B. Lot release and start of a stability program. An extensive array of assays developed and qualified in Aims 1 and 2 will establish criteria for the expression during formulation and manufacture and during stability (long-term and accelerated at different temperatures and during freeze/thaw). Quality control responsibilities will be performed by BCM-Sabin, the contract manufacturer and testing labs. BCM-Sabin will compile and generate the Certificate of Analyses (CoA) for the cell banks, drug substance and product.

3.C. IND Regulatory Package Preparation and Submission. A pre-IND briefing package including preclinical data, human immunoepidemiological data, and plans for GLP toxicology study. A concept clinical protocol will be submitted to the U.S. FDA after the release of the cGMP material. After the Pre-IND meeting and concurrence with our program plan and toxicology study design, a GLP study for rRBD and rRBD-based vaccine will be initiated with the selected AAALAC-accredited contract testing laboratory. Similar to other programs of BCM-Sabin, the ultimate intention is to have a completed IND package in order to advance into phase 1 clinical testing.

Human Subjects:

Human Subjects Involvement and Characteristics: Healthy human adult volunteer subjects are donors of serum specimen and convalescent serum specimens for recovered SARS patients with conformed specific antibody activity against SARS-CoV, a generous gift from our oversea collaborator, will be used for the *in vitro* studies to confirm the antigenicity of rRBD protein. The only use of human subjects for these studies is for the donated serum samples. Any healthy individual who, by virtue of anemia, might have additional risk (to those described below) from blood donation will be excluded. The healthy donors are commonly employees or students of the University of Texas Medical Branch at Galveston. (No student donors are required to participate, nor does student participation affect in any way evaluation or education.) Our volunteer donor pool will typically include Hispanic, African-American, and Asian (approximate male:female ratio of 1:1) individuals reflecting the population of Southeast Texas. Their age range will 18-55 years. Convalescent serum from three individuals recovered from SARS will be used.

Sources of Materials: Peripheral blood will be the only research material obtained from individually identifiable living human subjects. The peripheral blood will be obtained specifically for research purposes.

Potential Risks: Risks involved are those related to donation of 500 ml or less of blood and, therefore, are minimal. Criteria for advising of the risk of venipuncture have been formulated by a Human Use Committee 109 as follows: The risks of simple venipuncture include: Commonly, the occurrence of discomfort and/or bruise at the site of puncture; and less commonly, the formation of a small blood clot or swelling of the vein and surrounding issue, and bleeding from the puncture site.

Recruitment and Informed Consent: Subjects will be asked to donate blood. Risks for healthy subjects, as outlined above, are delineated by an investigator or by one of the trained designated research personnel, such that consent is informed. Such consent is documented by written confirmation, with the approval of the consent procedures and the written consent form by the Institutional Review Board of the University of Texas Medical Branch.

Protection Against Risk: Venipuncture will be performed by trained personnel in order to minimize the above risks. All blood samples are coded in the laboratory to establish donor confidentiality and records are kept in a locked cabinet in the Principal Investigator's locked laboratory.

Potential Benefits Of The Proposed Research To The Subjects And Others: There are no immediate or direct benefits to the volunteer subject. Financial reimbursement for time and effort is warranted and standard practice of our laboratory and will be given to the volunteers. Benefits to the volunteer and to society in general are those that come from extending medical knowledge of viral pathogenesis, with potential application to better management (prevention or therapy) of disease. The risks are limited in relation to the anticipated benefits to the subjects and to society.

INCLUSION OF WOMEN AND MINORITIES: Normal volunteers, including women and minorities, between the age range of 18 – 65 will be recruited to participate in this study (with the exception of pregnant females). Patients will be interviewed for any pre-existing conditions that may disqualify them as a blood donor. If the person(s) qualifies, a trained phlebotomist will conduct blood collection from the volunteers.

Program Director/Principal Investigator (Last, First, Middle):

Tseng, Chien-Te K ent PhD.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants.

Study Title: RBD recombinant protein-based SARS vaccine for biodefense (IRB-pending)

Total Planned Enrollment: 3

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Females	Males	Total
Hispanic or Latino			
Not Hispanic or Latino	1	2	3
Ethnic Category: Total of All Subjects *	1	2	3
Racial Categories			
American Indian/Alaska Native			
Asian	1	2	3
Native Hawaiian or Other Pacific Islander			
Black or African American			
White			
Racial Categories: Total of All Subjects *	1	2	3

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

INCLUSION OF CHILDREN: Children will NOT be included in these studies. As stated previously, we will **not** include any volunteer under the age of 18.

Vertebrate animal research:

1. Description of animal use. Both wild-type (*wt*) Balb/c mice and mice transgenically expressing hACE2, designated Tg⁺ mice, will be used for experiments to test the efficacy of the recombinant receptor binding domain (rRBD) subunit of SARS-CoV spike (S) protein to protect against lethal infection by mouse adapted SARS-CoV (MA-15) and homologous and heterologous strains of clinical isolate/s of SARS-CoV, respectively. Mice will be immunized according to the optimized vaccination regimens with regard to the dosing/frequencies, route/s of vaccination, intervals between vaccination, and the formulation of adjuvant/s, etc, prior to lethal challenge through intranasal (i.n.) infection with either MA-15 or clinical isolate/s of SARS-CoV in Balb/c and Tg⁺ mice, respectively. Some vaccinated and non-vaccinated mice will be sacrificed at various time points after viral infection for harvesting lungs and brain (for Tg⁺ mice only), for assessing viral yields, pathology, cytokine profiling, and IHC for assessing the protective efficacy. Other infected mice will be monitored daily for the clinical manifestations (e.g., weight loss, etc), and mortality (if any). Mice will be bled from the saphenous vein or via cardiac puncture (terminal bleeds only, under isoflurane anesthesia). Weights will be measured on a laboratory balance. This animal study will be conducted under approved ABSL-3 facilities at UTMB.

Species: *Mus musculus*

Strains: *Balb/c* mice will be obtained from the commercial sources, whereas Tg⁺ mice will be generated in house at UTMB by Dr. Chan, Tehsheng, Department of Microbiology and Immunology, UTMB.

Ages: 8-12 weeks

Sex: male and female

Numbers: Grand total of mice: N=420 (i.e., *Balb/c* mice: 300, Transgenic AC70 mice: 120)

Aim 1.D.2. Protection against SARS-CoV challenge.

■ Two treatments (i.e., vaccinated and non-vaccinated) X 15 *Balb/c* mice/cohort X 2 (repeat once) = 60 *Balb/c* mice.

Aim 1.F.2: Cross-protection.

■ Two different treatment (vaccinated and non-vaccinated) X 2 different viruses (i.e., homologous and heterologous) X 15 Tg⁺ mice/cohort X 2 (repeat once) = 120 Tg⁺ mice

Aim 1.G: Efficacy of the rRBD-based vaccine to induce long-term immune response and protection.

■ Two different treatment X three different time points X 20 *Balb/c* mice X 2 (repeat once) = 240 *Balb/c* mice

2. Justification: Both *Balb/c* mice and transgenic AC70 mice HACE2 transgenic mice are well-characterized models for mouse-adapted MA-15 SARS-CoV and clinical isolate/s of SARS-CoV infection, respectively, resulting in robust replication of SARS-CoV and onsets of diseases (e.g., weight loss), and mortality. In the absence of suitable *in vitro* or computer-based method that can be used to predict the protective efficacy of any given candidate vaccine against SARS-CoV infection, these mouse models are of valuable for reaching the goal of the proposed studies. The numbers to be used are the minimum required, bases on preliminary power analyses, to detect protective efficacy against illness and fatal disease of SARS-CoV infection.

3. Veterinary care: All animals will be housed in a veterinarian supervised, approved animal facilities that have been awarded Accreditation by the American Association for Accreditation of Laboratory Animal Care Council. The following statement explains UTMB's animal welfare compliance: "The University of Texas Medical Branch (UTMB) operates to comply with the USDA Animal Welfare Act (Public Law 89-544) as amended by PL91-579 (1970) PL94-279 (1976) and 45 CFR37618 (6-3-80); Health Research Extension Act of 1985 (Public Law 99-158); follows the Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised September 1986); and the Guide for the Care and Use of Laboratory Animals DHEW (NIH) 85-23 revised 1985. UTMB is a registered Research Facility under the Animal Welfare Act. It has a current Letter of Assurance on file with the Office of Protection from Research Risks, in compliance with NIH policy. The Animal Care Center is under the direction of a Doctor of Veterinary Medicine and staffed by veterinarians with training and experience in laboratory animal medicine and surgery, clinical care and diagnostic pathology. Routine veterinary care is provided for the animals in compliance with federal and state regulations. The Animal Care staff monitors the animals and the veterinarians personally make rounds each day, calling the investigator to ensure humanitarian care of all laboratory animals.

The animals are maintained in the UTMB Animal Care Facility at an ambient temperature of $30\pm 1^{\circ}\text{C}$ on a 12:12 hour light:dark cycle. Food is provided once daily in an amount sufficient for the cage containing no more than 5 mice, and water is provided *ad libitum*. Cages are cleaned on alternating days.

Housing and Care for infected mice: Infected animals will be housed in microisolator cages within the ABSL-2 or ABSL-3 and monitored daily for signs of disease associated with SARS-CoV infection. Blood samples will be taken via the saphenous vein, by or cardiac puncture (for terminal bleeds, under isoflurane anesthesia) to determine seroconversion. Analgesics cannot be used because they might have unintended physiological effects that would influence pathogenesis and the ability to detect protective effects of the vaccines.

4. Procedures for ensuring that discomfort, distress, pain, and injury. The goal of this project is to evaluate rRBD-based lead candidate vaccine against SARS-CoV infection and associated diseases and mortality. Unfortunately, it is not possible to use moribund endpoints in this study because we need to determine if the vaccine will protect against a lethal outcome, in addition to disease and viremia. It would be impossible to interpret our results if additional anti-inflammatory or any other analgesic drugs were used. Also, all analgesics interact in some way with the inflammatory response, which is tied to pathogenesis, and some can affect the blood brain barrier, which could also affect pathogenesis.

5. Euthanasia: Mice will be euthanized with an intravenous overdose of pentobarbital, or by expos

Multiple PD/PI Leadership Plan

Administration: This proposal involves the collaboration of Dr. Peter Hotez and Dr. Maria Elena Bottazzi at the Department of Pediatrics, Baylor College of Medicine (the new home of Sabin Vaccine Institute's product development partnership (BCM-Sabin), and Dr. Shibo Jiang and Dr. Sara Lustigman at the Lindsley F. Kimball Research Institute of the New York Blood Center (NYBC). Due to the complexities and broad scope of the proposed research, successful completion will require diverse areas of expertise and interests as represented by the Principal Investigators (PIs) and their laboratories. Since the success of this application depends on critical contributions from each of the named PIs, a multi-PI model is the best fit for this project. The responsibility for the named PIs is described below and each PI will be responsible for her/his own fiscal and research management.

PI#1 Peter Hotez, MD, Ph.D. Professor, Department of Pediatrics, Baylor College of Medicine; President, Sabin Vaccine Institute. He is a laboratory and clinician investigator with expertise in recombinant protein vaccines for neglected tropical diseases (NTDs). He is an elected member of the Institute of Medicine and founding director of one of the first non-profit public private partnerships (PDP) for developing vaccines to combat NTDs. He will be the PI#1 and the point of contact for the project. He works closely with PI# 3 to lead all the product development activities and will interact with PI#2 and Dr Sara Lustigman and Chien-Te K. Tseng at the Department of Microbiology and Immunology, University of Texas Medical Branch (UTMB) for the progress of the Aims.

PI#2 Shibo Jiang, M.D., Ph.D., Member and Head of the Viral Immunology Laboratory, Lindsley F. Kimball Research Institute of New York Blood Center. He is a well-established virologist and immunologist with medical background. He has extensive experience in the development of vaccines against SARS, influenza and HIV/AIDS. **PI#2** will plan, direct and execute the majority of the studies in Aim 1. He will supervise Dr. Lanying Du (Co-investigator) and Dr. Peng Zou (Postdoctoral Research Fellow), and participate in the related experiments, evaluate the experimental results, and the writing of progress reports and papers. He will interact with **PI#1**, **PI#3** and Dr. Sara Lustigman (NYBC), and be the liaison for the efficacy studies in collaboration with Dr. Chien-Te K. Tseng (UTMB).

PI#3 Maria Elena Bottazzi, Ph.D. Professor, Department of Pediatrics, Baylor College of Medicine; Director for Product Development, Sabin Vaccine Development. Her major expertise is in translational research and vaccine development for neglected tropical diseases and the role of vaccines as control tools in international public health programs and initiatives. Dr. Bottazzi will supervise all the expression process/analytical development and technology transfer activities. She will interact with Dr Sara Lustigman, who will be the NYBC liaison for all aspects involving the product development of recombinant RBD and adjuvant formulation.

Communication: The PIs, key personnel, and the subcontractors will have bi-monthly phone or web-conferences through Skype (<http://www.skype.com/intl/en-us/features/allfeatures/conference-calls>) or Arkadin Global Conferencing service (<https://www.anywhereconference.com>) to discuss experimental design, data analysis, and updates regarding administrative and regulatory responsibilities. PIs, key personnel, and the subcontractors will meet annually for a one day meeting to formally present data, and discuss scientific and administrative issues. PIs will share their respective research results with each other, and with the key personnel, subcontractors and consultants. They will work together to discuss any changes in the direction of the research projects and the reallocation of funds, if necessary. Dr. Hotez (**PI#1**) will serve as contact PI and be responsible for the submission of the annual progress report to NIH and all communications with the sponsoring organization.

Publication and Intellectual Property: Publication authorship and patent inventorship will be determined based on the relative scientific contributions of the PIs and key personnel.

Conflict Resolution: If a potential conflict develops in connection with this grant, the PIs shall first meet for attempted resolution in good faith within thirty (30) days of the notice of such dispute. If they fail to resolve the dispute, the disagreement may be referred to any dispute resolution mechanism that the institution chooses, including an arbitration committee consisting of senior executives mutually agreed upon by all PIs. No members of the arbitration committee will be directly involved in the research grant.

Change in PI Location: If a PI moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution.

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COLLABORATIVE/CONTRACTUAL ARRANGEMENTS

Sub-awards

Two organizations will be subawarded: New York Blood Center (NYBC) and University of Texas Medical Branch (UTMB).

At **NYBC**, Dr. Shibo Jiang, Head of Viral Immunology Laboratory and Sara Lustigman, Head of the Laboratory of Molecular Parasitology will plan, direct and execute the majority of the studies in Aim 1 in close involvement and discussion with Dr Hotez and Dr Bottazzi at BCM. NYBC will also be the liaison for the efficacy studies in collaboration with Dr. Chien-Te K. Tseng at the Department of Microbiology and Immunology, University of Texas Medical Branch (UTMB).

At **UTMB**, Dr Chien-Te K. Tseng, PhD, Associate Professor, Department of Microbiology and Immunology, Center for Biodefense and Emerging Diseases, Institute of Human Infection and Immunity, Sealy Center for Vaccine Development, and Galveston National Laboratory (GNL) at UTMB-Galveston, will serve as principal investigator. Dr. Tseng's will focus on defining the host innate antiviral responses against and the pathogenesis of the severe acute respiratory syndrome (SARS) coronavirus (CoV). Dr. Tseng will be the lead investigator in SARS pathogenesis with the animal model for the SARS research, including several strains of gene knockout (KO) mice and wild-type (wt) mice. Dr. Tseng will be fully engaged in all phases of this project, including coordinating, planning and designing, and together with his staff, executing and supervising the proposed experiments. He will also be responsible for the interpretation of the data, preparing the reports and manuscripts as required for publications.

The allocations for these sub-grants are as follows:

Organization	Direct Costs per year				
	Year 1	Year 2	Year 3	Year 4	Year 5
NYBC	\$275,000	\$ 300,000	\$ 175,000	\$ 75,000	\$ 50,000
UTMB	\$125,000	\$ 200,000	\$ 150,000	\$ 0	\$ 0

Contracts

Baylor College of Medicine will enter into a contractual agreement with **Sabin Vaccine Institute** for activities to be performed under Specific Aim 2 and 3. A fee-for-service contract will be provided to the Sabin Vaccine Institute to cover activities related to Quality assurance, regulatory and to manage the contract cGMP manufacturing, GLP toxicology and purchase of the adjuvant. The contract will be distributed as follows: Year 1: \$75,000; Year 2: \$575,000 and Year 3: \$600,000

Sabin Vaccine Institute will manage the fee-for-service contracts and activities related to the GMP manufacture and the GLP toxicology testing for the RBD recombinant protein based vaccine for SARS. In addition, it will implement its Quality Management System (QMS) to provide quality assurance (QA) function for the process development, manufacturing, and toxicology testing activities as outlined in the grant proposal.

This system includes the operation of:

- Policy of compliance with applicable regulations and ethical obligations
- Standard Operating Procedures to assure the standardization of relevant procedures, which includes, but is not limited to, laboratory techniques, documentation practices, and the maintenance of laboratory equipment.
- Documentation system which enables the efficient origination, completion, and storage of documents that contain information on both regulated activities and other activities that are conducted in support of downstream product manufacture
- Conduct of Quality Assurance (QA) audits
- Program for risk assessment and risk management

Finally Sabin will purchase the toxicology-grade aqueous or stable emulsion formulation of Gluco-Pyranosylphospho-Lipid A (GLA-AF, a synthetic lipid A derivative), an immunostimulant for use in the GLP toxicology study from Immune Design Corporation (I DC).

△ New York Blood Center
Lindsley F. Kimball Research Institute

Office of Sponsored Programs
310 East 67 Street.
New York, NY 10065-6275
Phone: 212-570-3034
Fax: 212-737-4506
Email: bgreene@nybloodcenter.org

May 10th, 2011

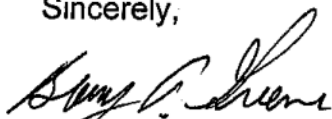
Baylor College of Medicine
Department of Pediatrics
6621 Fannin
Suite A150, MC 1-3420
Houston, TX 77030

Re: Statement of Intent to Establish a Consortium Agreement with Baylor College of Medicine

This letter is to confirm that the New York Blood Center agrees to participate in the study entitled "RBD recombinant protein-based SARS vaccine for biodefense preparedness". The principal investigator for the New York Blood Center is Dr. Shibo Jiang. The total costs for New York Blood Center will be \$1,436,750, for a Period of Performance of May 1st, 2012 – April 30th, 2017. This includes the on-site facilities and administrative costs of 64.2%. The DUNS number for the New York Blood Center is 073271827.

The appropriate programmatic and administrative personnel of Baylor College of Medicine will establish written inter-institutional agreements to ensure compliance with all pertinent federal regulations and policies in accordance with the *PHS Grants Policy Statement*, *PHS 398 Application for Public Health Service Grant*, and the *NIH Guidelines for Establishing and Operating Consortium Grants*.

Sincerely,



Barry A. Greene, M.P.A.
Executive Director
Office of Sponsored Programs



301 University Blvd.
Galveston, TX 77555-0150

May 10, 2011

Baylor College of Medicine
Department of Pediatrics
6621 Fannin, Suite A150, MC 1-3420
Houston, TX 77030

Re: Statement of Intent to Establish a Consortium Agreement with Baylor College of Medicine

This letter is to confirm that the University of Texas Medical Branch agrees to participate in the study entitled "*RBD recombinant protein-based SARS vaccine for biodefense preparedness*.. The principal investigator for UTMB is Chiente Tseng, Ph.D. The total costs for UTMB will be \$726,750 for a Period of Performance of 5-1-12 through 4-30-16. This includes the off-campus facilities and administrative costs of 53%. The DUNS number for UTMB is 8007711490000..

The appropriate programmatic and administrative personnel of Baylor College of Medicine will establish written inter-institutional agreements to ensure compliance with all pertinent federal regulations and policies in accordance with the *PHS Grants Policy Statement*, *PHS 398 Application for Public Health Service Grant*, and the *NIH Guidelines for Establishing and Operating Consortium Grants*.

Sincerely,

A handwritten signature in cursive script that reads "Connie Barton".

Connie Barton
Associate Director, Office of Sponsored Programs



May 5, 2011

Baylor College of Medicine
Department of Pediatrics
6621 Fannin, Suite A150, MC 1-3420
Houston, TX 77030

Re: Letter of Support in response to NIH/NIAID Partnerships for Biodefense (R01) RFA-AI-11-014
Regarding Contract The Sabin Vaccine Institute.

This letter is to confirm that The Sabin Vaccine Institute agrees to participate in the study entitled "RBD recombinant protein-based SARS vaccine for biodefense."

As part of its scope of work, The Vaccine Development Team at the Sabin Vaccine Institute (Sabin) will perform three main functions. First, Sabin will manage the fee-for-service contracts and activities related to the GMP manufacture of and the GLP toxicology testing for the RBD recombinant protein-based vaccine for SARS. Second, Sabin will implement its Quality Management System (QMS) to provide quality assurance (QA) function for the process development, manufacturing, and toxicology testing activities as outlined in the grant proposal. And third, Sabin will purchase the toxicology-grade aqueous or stable emulsion formulation of Gluco-Pyranosylphospho-Lipid A (GLA-AF, a synthetic lipid A derivative), an immunostimulant for use in the GLP toxicology study from Immune Design Corporation (IDC).

The Vaccine Development Team at Sabin currently manages sub-grantees on five continents to support the development of vaccines for human hookworm and schistosomiasis. These include academic collaborators, governmental entities (in Brazil and China), and fee-for-service contractors. Sabin has successfully managed and completed three GMP manufacturing campaigns and three full GLP toxicology studies including study design and sponsor-level QA.

The Vaccine Development Team at Sabin has installed and manages a fully operational Quality Management System (QMS) to ensure that all work completed at the Baylor College of Medicine/Texas Children's Hospital and other partners adhere to established benchmarks of quality. This system includes the operation of:

- Policy of compliance with applicable regulations and ethical obligations
- Standard Operating Procedures to assure the standardization of relevant procedures, which includes, but is not limited to, laboratory techniques, documentation practices, and the maintenance of laboratory equipment.
- Documentation system which enables the efficient origination, completion, and storage of documents that contain information on both regulated activities and other activities that are conducted in support of downstream product manufacture
- Conduct of Quality Assurance (QA) audits
- Program for risk assessment and risk management

We understand that the appropriate programmatic and administrative personnel of Baylor College of Medicine will establish written inter-institutional agreements to ensure compliance with all pertinent federal regulations and policies in accordance with the PHS Grants Policy Statement, PHS 398

Page 2 of 2

Application for Public Health Service Grant, and the NIH Guidelines for Establishing and Operating Consortium Grants.

The attached proposal estimates the costs necessary to implement the project.

Sincerely,

(b)(6)

Brian R. Davis
Chief Operating Officer

Phone: (b)(6)
Email:



May 5, 2011

Baylor College of Medicine
Department of Pediatrics
6621 Fannin, Suite A150, MC 1-3420
Houston, TX 77030

Re: Letter of Support in response to NIH/NIAID Partnerships for Biodefense (R01) RFA-AI-11-014
Regarding Contract The Sabin Vaccine Institute (Sabin)

Thank you for the opportunity to submit a cost proposal for Sabin's services in support of the grant proposal entitled, "RBD recombinant protein-based SARS vaccine for biodefense."

Activity	Year 3 (05/14-04/15)	Year 4 (05/15-04/16)	Year 5 (05/16-04/17)	Total
Sabin Services:				
Quality assurance and regulatory support for process development	\$75,000			\$75,000
Quality assurance, management and oversight of GMP manufacturing campaign		\$51,000		\$51,000
Quality assurance, management and oversight of GLP Toxicology Study			\$238,550	\$178,550
Contracted Services:				
GMP Manufacturing Campaign		\$524,000		\$524,000
GLP Toxicology Study			\$186,450	\$186,450
Glucopyranosylphospho-Lipid A (GLA, a synthetic lipid A derivative)			\$175,000	\$235,000
Total:	\$75,000	\$575,000	\$600,000	\$1,250,000

Sincerely,

(b)(6)

Brian R. Davis
Chief Operating Officer

Phone: (b)(6)

Email:

4 May 2011

Michael W. Marine
Chief Executive Officer
Sabin Vaccine Institute
2000 Pennsylvania Avenue, Suite 7100
Washington, DC 20006

(b)(6)

Dear Mr. Marine:

COUNTERSIGNED LETTER OF COMMITMENT - RFA-AI-11-014

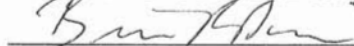
In response to the NIH/NIAID Partnerships for Biodefense (R01) RFA-AI-11-014, I write to advise that I am delighted to be involved as a consultant in support of the project entitled "**RBD recombinant protein-based SARS vaccine for biodefense preparedness**" that is currently being prepared for submission by the Sabin Vaccine Institute. I will serve as a Quality Assurance consultant for preclinical activities in support of Sabin Vaccine Institute's vaccine development programs undertaken at the Baylor College of Medicine/Texas Children's Hospital or other locations as needed. In this capacity, I will assess and monitor the project in compliance with Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) and conduct periodic inspections, data audits, protocol reviews, write and revise standard operating procedures (SOPs), reports, and other quality assurance-related activities upon request. The consulting rate will be \$90.00 per hour with a maximum of twenty (20) hours per week plus mileage expenses as allowed by IRS, or other transportation expenses incurred for travel to and from required worksites.

Yours sincerely,

(b)(6)

LINDA SCHELLHAAS, BA, RQAP-GLP
QUALITY REVIEWS, INC.
130 TRAVELLER ROAD
FALLING WATERS, WV 25419

With the concurrence of The Sabin Vaccine Institute:



Brian R. Davis
Chief Operating Officer



DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
503 ROBERT GRANT ROAD
SILVER SPRING, MD 20910-7500

28 Apr 11

Mr. Michael W. Marine
Chief Executive Officer
Sabin Vaccine Institute
2000 Pennsylvania Avenue NW
Suite 7100
Washington, DC 20006

Re: Letter of Support for the WRAIR Pilot Bioproduction Facility to act as cGMP manufacturer for a recombinant protein-based SARS vaccine identified in NIH RFA-AI-11-014: Partnerships for Biodefense

Dear Mr. Marine:

Walter Reed Army Institute of Research (WRAIR), Pilot Bioproduction Facility (PBF), is pleased to provide this Letter of Support for inclusion in the Sabin Vaccine Institute proposal to develop and test a recombinant protein-based SARS vaccine. As part of the development and testing regimen described in the proposal, WRAIR-PBF is willing and able to perform cGMP manufacturing services for the preparation of the vaccine. Attached is a scope of work proposed for the vaccine including an itemized cost estimate based on current (CY11) costs. These services will be provided as part of a Cooperative Research and Development Agreement between WRAIR and Sabin Vaccine Institute post award of the grant. The CRADA specifies patent and publication rights for the client and the government. This preliminary Letter of Commitment is non-binding and preliminary to the negotiation of a final agreement with the Sabin Vaccine Institute. Also attached is background information on the mission of the PBF.

Best Regards,

(b)(6)

Kenneth H. Eckels, PhD
Chief, Pilot Bioproduction Facility
WRAIR

With the concurrence of the Sabin Vaccine Institute:

(b)(6)

Brian R. Davis
Chief Operating Officer

Vaccine Production and Costs

Production Item	Cost K\$*
Master and Working Cell Banking	80
Fermentation	180
Purification	220
Fill	44
TOTAL	524

*CY 11 costs

Scope of Work

1. Prepare under GMP conditions Master and Working cell banks for SARS recombinant (yeast, bacteria); 100 vials of each.
2. Ferment under GMP condition up to 300 L of SARS recombinant (yeast or bacteria); process concentrated bacterial paste or supernatant fluids from yeast.
3. Purify under GMP conditions an aliquot of SARS recombinant gene product to prepare bulk vaccine.
4. Fill under GMP conditions bulk vaccine into vaccine vials, up to 2,000 vials.
5. Lab-scale processes for all of the above will be provided to the PBF.
6. A research-grade construct seed will be provided to the PBF for cell banking.

Pilot Bioproduction Facility, WRAIR

Established in 1958 as the Department of Biologics Research, the Pilot Bioproduction Facility (PBF) has specialized in developing vaccines for DOD mission-related disease threats. More recently, projects for public and private partners have been accomplished through inter-agency and cooperative agreements. The PBF's mission is research, development, production, and testing of vaccines for human use. The PBF at Forest Glen, MD is a multi-use facility designed and operated for production of vaccines following current Good Manufacturing Practices (cGMP) regulations. Compliance with cGMP ensures that products prepared in the facility will be safe, potent, and reproducible.

PBF Successes

In the facility's distinguished history, the PBF has received awards and accolades for its role in developing vaccines for deadly diseases like hepatitis A, Japanese encephalitis, and meningitis. More recently, the facility has played a key role in the development of vaccines for dengue fever, malaria, HIV, cholera, and shigellosis. Many of these experimental vaccines have progressed to advanced clinical testing.



May 9, 2011

Mr. Michael W. Marine
Chief Executive Officer
Sabin Vaccine Institute
2000 Pennsylvania Avenue NW, Suite 7100
Washington, DC 20006

RE: Letter of Support in response to NIH/NIAID Partnerships for Biodefense (R01)
RFA-AI-11-014 Regarding Subcontract with Frontier BioSciences, Inc. ("Frontier
BioSciences")

Project Title: RBD Recombinant Protein-based SARS Vaccine for Biodefense Preparedness

Dear Mr. Marine:

Frontier BioSciences would be happy to participate in the above-referenced project. We look forward to working closely with you and your staff. We will ensure high quality deliverables and flexibility to your program requirements, and will work effectively with your team to meet your proposed timeline for your program. We are fully prepared to establish the necessary agreements and contracts if this proposal is funded.

The attached proposal estimates the cost necessary to implement the project.

Please feel free to contact me if you require other information or have any questions regarding Frontier Bioscience's participation. Please notify us if the project is funded, so that we may implement a subcontract without delay.

Thank you for the opportunity to be involved in this important project.

(b)(6)

05/09/11

Elizabeth C. Wong
Vice President, Business Development

Date

Contact Information:

Phone (b)(6)
Cell (b)(6)
Email (b)(6)

With the concurrence of The Sabin Vaccine Institute:

(b)(6)

Brian R. Davis
Chief Operating Officer



May 9, 2011

Mr. Michael W. Marine
Chief Executive Officer
Sabin Vaccine Institute
2000 Pennsylvania Avenue NW, Suite 7100
Washington, DC 20006

Re: RBD Recombinant Protein-based SARS Vaccine for Biodefense Preparedness

Dear Mr. Marine:

Thank you for the opportunity to provide a proposal for your **RBD Recombinant Protein-based SARS Vaccine for Biodefense Preparedness**.

Included in this proposal is the study outline provided by Sabin. Our cost estimate was based upon the details included in this outline.

The final pricing may vary upon the finalization of the protocol, as well as consideration of other components not included in this proposal.

RBD Recombinant Protein-based SARS Vaccine for Biodefense Preparedness

Species	Rat
GLP Status	GLP
Acclimation Period	1 week
In Life Period	9 weeks, not including 1-week acclimation period
Recovery Period	2 weeks
No. Animals/Sex/Dose Group	Main – 10/sex/group Satellite – 5/sex/group
No. of Dose Groups (including control)	7 dose groups, including control
Total number of animals on study	210
Dose Formulation Analysis	Performed by Sabin
Formulations	Dose prep prior to dosing (4 dose timepoints)
Dose Frequency	Once on SD0, 21, 42 and 63
Route	Intramuscular
Cage side observations	At least 2 times/day
Clinical Observations	During quarantine, and prior to dosing on SD 0, 21, 42 and 63 and 1 – 3 hours post dose; Once daily on Study Days 1 through 7 and weekly thereafter throughout the remainder of the in-life study.
Body Weights	Prior to randomization, and prior to dosing on days SD0, 21, 42 and 63 and on Study Days 2, 4, 6, 8, 15, 29, 36, 50, 57, 66, 71



RBD Recombinant Protein-based SARS Vaccine for Biodefense Preparedness

	and 77. Fasted body weights will also be collected from all surviving main study animals prior to their scheduled sacrifice on Study Day 67 or 78.
Body Temperature	Body temperature will be determined prior to each dose, 6 hours (± 10 minutes) and 24 hours (± 20 minutes) post dosing. If any temperature is $> 40^{\circ}\text{C}$, additional measurements will be taken every 24 hours until the values return to normal.
Food Consumption	Food consumption will be measured individually for each surviving main study animal weekly throughout the treatment and observation periods.
Draize Scoring	Inoculation sites from all main study animals will be evaluated prior to dosing on Study Day 0, 21, 42, and 63 and on SD 1-7; SD22-28; SD43-49 and SD64-70 and prior to termination on SD 67 or 78.
Clinical Chemistry	During pre-test period and on Study Day 4 and prior to dosing on Study Days 22, 43 and 64, and prior to termination on Study Day 67 or 78. (5 rats/sex/group/time point).
Hematology	During pre-test period and on Study Day 4 and prior to dosing on Study Days 22, 43 and 64, and prior to termination on Study Day 67 or 78. (5 rats/sex/group/time point).
Coagulation Parameters	During pre-test period and on Study Day 4 and prior to dosing on Study Days 22, 43 and 64, and prior to termination on Study Day 67 or 78. (5 rats/sex/group/time point).
Urinalysis	N/A
Antibody Sample Collection	From Satellite animals, up to 5 animals/sex/group, on Study Day 1, 22, 43, and 64 prior to dosing, and from Main Study animals, SD 67 (terminal deaths) and from Main Study and Satellite animals up to 10 animals/sex/group, on day 78.
Necropsy/Tissue preservation	Tissues from all Main Study animals will be collected at necropsy and fixed in 10% neutral buffered formalin, with the exception of the eyes (with optic nerves) which will be fixed in Davidson's solution, and the testes and epididymides which will be fixed in Bouin's fixative.
Bone Marrow	Collected and fixed in methanol. Evaluated upon request at additional charge.
Histopathology	Main study animals in Groups 2, 4 and 6 only. Additional groups evaluated at additional charge.
Report	Standard GLP Report

Cost: \$186,450



We understand that this project is scheduled for 2016/2017. Please let us know when the project is funded so we can get the appropriate paperwork in place and assign the resources.

We look forward to working closely with you and your staff. We will ensure high quality deliverables and flexibility to your program requirements, and will work effectively with your team to meet your proposed timeline for your program.

Should you have any questions regarding this proposal, please do not hesitate to call me.

Best regards,

(b)(6)

Beth Wong, VP Business Development



May 12, 2011

Mr. Michael Marine
Chief Executive Office
Sabin Vaccine Institute
2000 Pennsylvania Ave NE
Washington DC 20006

RE: "Grant RBD recombinant protein-based SARS vaccine for biodefense preparedness' in response to NIH's RFA for Partnerships for Biodefense".

Dear Mr. Marine:

The purpose of this letter is to convey Immune Design Corp.'s (IDC) support for your grant application entitled, "RBD recombinant protein-based SARS vaccine for biodefense preparedness' in response to NIH's RFA for Partnerships for Biodefense."

If the grant is funded, and pending execution of an MTA between IDC and the Sabin Vaccine Institute, we will supply you with our Glycopyranosyl Lipid Adjuvant (GLA, aqueous and stable emulsion formulations), a TLR4 agonist, for use in feasibility studies and the GLP toxicology study as detailed in your grant application. The current cost the formulated GLA well be approximately \$175,000.

We look forward to working with you on this important project.

Sincerely,

(b)(6)

Steven G. Reed, Ph.D.
President

With the concurrence of The Sabin Vaccine Institute:

(b)(6)

Brian R. Davis
Chief Operating Officer

1124 COLUMBIA STREET, SUITE 700
SEATTLE, WA 98104
P: 206.682.0645 F: 206.682.0648
INFO@IMMUNEDESIGN.COM

Resource Sharing Plan

We will adhere to the NIH Grant Policy regarding the sharing of unique research resources developed through NIH-sponsored research, including research data and reagents. Data files will be made available to outside researchers in a timely fashion - no later than the acceptance for publication of the main findings reported in individual manuscripts. Requests for reagents that are generated as part of this project will be distributed in a timely manner. Distribution to academic and other non-profit researchers will occur under agreements no more restrictive than the Materials Transfer Agreement of the Baylor College of Medicine and the New York Blood Center or the Simple Letter Agreement for the Transfer of Materials as described in the Guidelines for Disseminating Research Resources Arising Out of NIH-Funded Research (Federal Register, Vol 64, No 246, p.72094). Distribution of resources to for-profit institutions will be determined based on the resource itself, the ability of the Baylor College of Medicine and the New York Blood Center to efficiently meet demand, and the necessity for further development of the resource. Distribution to for-profit entities may occur under Material Transfer agreements or non-exclusive license arrangements. Only under extreme circumstances would exclusive licensing be considered; The offices of Patents & Licensing of the Baylor College of Medicine and the New York Blood Center will contact NIH's technology transfer office to discuss the need for exclusive licensing of research resources, should the Baylor College of Medicine and the New York Blood Center believe this is required for adequate distribution of the resource to both for-profit and non-profits.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

* First Name:

Middle Name:

* Last Name:

Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$)

*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☐ Yes ☒ No



Grant Number: 3R01AI098775-02S1

Principal Investigator(s):

Maria Elena Bottazzi
PETER J HOTEZ (contact)
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Leanne Brooks Scott
Business Official
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Budget Period: 06/24/2013 – 08/02/2013

Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$3,936 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Shellie M. Wilburn

Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 3R01AI098775-02S1**Award Calculation (U.S. Dollars)**

Salaries and Wages	\$2,320
Fringe Benefits	\$182
Federal Direct Costs	\$2,502
Federal F&A Costs	\$1,434
Approved Budget	\$3,936
Federal Share	\$3,936
TOTAL FEDERAL AWARD AMOUNT	\$3,936
AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$3,936

SUMMARY TOTAL FEDERAL AWARD AMOUNT YEAR (2)	
GRANT NUMBER	TOTAL FEDERAL AWARD AMOUNT
3R01AI098775-02S1	\$3,936
5R01AI098775-02	\$1,085,321
TOTAL	\$1,089,257

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
2	\$3,936	\$1,089,257
3	\$0	\$1,134,359
4	\$0	\$1,165,726
5	\$0	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number: 93.855
EIN: 1741613878A1
Document Number: RAI098775A
Fiscal Year: 2013

IC	CAN	2013
AI	8012165	\$3,936

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC: M51C B / OC: 414C / Released: PII 06/21/2013
Award Processed: 06/24/2013 12:04:45 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 3R01AI098775-02S1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 3R01AI098775-02S1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- The grant program legislation and program regulation cited in this Notice of Award.
- Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- 45 CFR Part 74 or 45 CFR Part 92 as applicable.

- d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at '<http://grants.nih.gov/grants/policy/awardconditions.htm>' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

Treatment of Program Income: Additional Costs

SECTION IV – AI Special Terms and Conditions – 3R01AI098775-02S1

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

This award provides support under the Research Supplements to Promote Diversity in Health-Related Research Program for Frederick Nelson /BAYLOR COLLEGE OF MEDICINE beginning 06/10/2013. These funds (\$2,502 Direct Costs and \$1,434 Facilities and Administrative Costs) are restricted for the above purpose only and may not be transferred to any other individual. Funds awarded are available for carryover for awards given carryover authority as reflected in section III of this award notice. However, the funds remain restricted for the individual and the purpose for which the supplement is awarded.

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)

(<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Vandhana Khurana
Email: khuranav@mail.nih.gov **Phone:** 301-496-7075 **Fax:** 301-493-0597

Program Official: Rachelle Salomon
Email: salomonra@mail.nih.gov **Phone:** 301-402-2202 **Fax:** 301-496-8030

SPREADSHEET SUMMARY

GRANT NUMBER: 3R01AI098775-02S1

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

<i>Budget</i>	<i>Year 2</i>	<i>Year 3</i>	<i>Year 4</i>	<i>Year 5</i>
Salaries and Wages	\$2,320			
Fringe Benefits	\$182			
TOTAL FEDERAL DC	\$2,502			
TOTAL FEDERAL F&A	\$1,434			
TOTAL COST	\$3,936	\$0	\$0	\$0

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	57.3%			
F&A Cost Base 1	\$2,502			
F&A Costs 1	\$1,434			

Department of Health and Human Services Public Health Services Grant Application <i>Do not exceed character length restrictions indicated.</i>		LEAVE BLANK—FOR PHS USE ONLY.			
		Type	Activity	Number	
		Review Group		Formerly	
		Council/Board (Month, Year)		Date Received	

1. TITLE OF PROJECT <i>(Do not exceed 81 characters, including spaces and punctuation.)</i> RBD RECOMBINANT PROTEIN-BASED SARS VACCINE FOR BIODEFENSE -1R01AI098775-01					
2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES <i>(If "Yes," state number and title)</i> Number: PA-12-149 Title: Research Supplements to Promote Diversity in Health Related Research					
3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR					
3a. NAME (Last, first, middle) Peter Hotez		3b. DEGREE(S) MD PhD		3h. eRA Commons User Name eRA Commons User Name	
3c. POSITION TITLE Professor		3d. MAILING ADDRESS <i>(Street, city, state, zip code)</i> One Baylor Plaza BCM 113 Houston, TX 77030-3411			
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Pediatrics, Tropical Medicine					
3f. MAJOR SUBDIVISION College of Medicine					
3g. TELEPHONE AND FAX <i>(Area code, number and extension)</i> TEL: 713-798-1199 FAX: 713-798-2299		E-MAIL ADDRESS: hotez@bcm.edu			
4. HUMAN SUBJECTS RESEARCH ☒ No ☐ Yes		4a. Research Exempt ☐ No ☐ Yes		If "Yes," Exemption No.	
4b. Federal-Wide Assurance No.		4c. Clinical Trial ☐ No ☐ Yes		4d. NIH-defined Phase III Clinical Trial ☐ No ☐ Yes	
5. VERTEBRATE ANIMALS ☒ No ☐ Yes			5a. Animal Welfare Assurance No.		
6. DATES OF PROPOSED PERIOD OF SUPPORT <i>(month, day, year—MM/DD/YY)</i> From 06/10/13 Through 08/02/13		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD 7a. Direct Costs (\$) \$2,502		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT 7b. Total Costs (\$) \$3,936 8a. Direct Costs (\$) \$2,502 8b. Total Costs (\$) \$3,936	
9. APPLICANT ORGANIZATION Name Baylor College Of Medicine Address One Baylor Plaza Houston, TX. 77030-3411		10. TYPE OF ORGANIZATION Public: → ☐ Federal ☐ State ☐ Local Private: → ☒ Private Nonprofit For-profit: → ☐ General ☐ Small Business ☐ Woman-owned ☐ Socially and Economically Disadvantaged			
		11. ENTITY IDENTIFICATION NUMBER 1741613878A1 DUNS NO. 051113330 Cong. District TX-009			
12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE Name Leanne B. Scott, Ph.D. Title Director, Sponsored Programs Address One Baylor Plaza BCM 310 Houston, Texas 77030-3411 Tel: 713.798.1297 FAX: 713.798.6990 E-Mail: spo@bcm.edu		13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION Name Leanne B. Scott, Ph.D. Title Director, Sponsored Programs Address One Baylor Plaza BCM 310 Houston, Texas 77030-3411 Tel: 713.798.1297 FAX: 713.798.6990 E-Mail: spo@bcm.edu			
14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.					DATE 4-15-13

PROJECT SUMMARY (See instructions):

Funds are requested for a minority supplement to the parent grant AI-098775 on the development and manufacture of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV). The project will serve as a basis for engaging an under-represented minority high school student in an eight-week long mentored program of molecular biology and biochemistry research. The program will be offered in association with the Office of Diversity and Community Outreach at Baylor College of Medicine.

Briefly, the parent grant seeks to develop a recombinant vaccine against SARS, now classified by NIAID as a Category C Priority Pathogen, by advancing the vaccine through early stage expression and preclinical characterization, process development, formulation, stability, technology transfer and cGMP manufacture. The Actual Specific Aims of the parent grant are as follows:

Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate.

Specific Aim 2: Process development, characterization, formulation and stability profiling. In parallel to Aim 1, a scalable and reproducible fermentation process for rRBD (10 liter scale) and a purification process using chromatographic technologies will be developed.

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation.

With the exception of the final cGMP manufacture and preclinical testing for efficacy using a mouse challenge model all of the work is conducted at the Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development of Baylor College of Medicine.

RELEVANCE (See instructions):

Under the guidance of an experienced researcher the minority student will learn fundamental techniques in molecular biology and biochemistry. These studies will be conducted in the larger context and environment of the steps needed to develop and test recombinant protein vaccines for important infectious agents and biodefense threats including SARS. Thus we hope to stimulate the imagination of the trainee about the role he/she could one day have in the important area of public health emergency preparedness.

PROJECT/PERFORMANCE SITE(S) (if additional space is needed, use Project/Performance Site Format Page)

Project/Performance Site Primary Location			
Organizational Name: Baylor College of Medicine			
DUNS: 051113330			
Street 1: One Baylor Plaza		Street 2:	
City: Houston	County: Harris	State: TX	
Province:	Country: United States of America	Zip/Postal Code: 77030	
Project/Performance Site Congressional Districts: TX-009			
Additional Project/Performance Site Location			
Organizational Name: Texas Children's Hospital			
DUNS: 074615394			
Street 1: 1102 Bates Ave. Ste 550		Street 2:	
City: Houston	County: Harris	State: TX	
Province:	Country: United States of America	Zip/Postal Code: 77030	
Project/Performance Site Congressional Districts: TX-009			

Program Director/Principal Investigator (Last, First, Middle): **Hotez, Peter**

SENIOR/KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Program Director(s)/Principal Investigator(s). List all other senior/key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Peter Hotez, MD, PhD	eRA Commons User Name	BCM	PI

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project
James L. Phillips, MD	BCM	Other Significant Contributor

Human Embryonic Stem Cells ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: <http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp>. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

**DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY**FROM
06/10/2013THROUGH
08/02/2013List PERSONNEL (*Applicant organization only*)

Use Cal, Acad, or Summer to Enter Months Devoted to Project

Enter Dollar Amounts Requested (*omit cents*) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnth	Acad. Mnth	Summer Mnth	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Peter Hotez, MD, PhD	PD/PI	0				0	0	0
Frederick Nelson	Project Intern	EFFORT			Institutional Base Salary	2,320	182	2,502
SUBTOTALS →						2,320	182	2,502

CONSULTANT COSTS

EQUIPMENT (*Itemize*)SUPPLIES (*Itemize by category*)

TRAVEL

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS (*Itemize by category*)OTHER EXPENSES (*Itemize by category*)

CONSORTIUM/CONTRACTUAL COSTS

DIRECT COSTS

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (*Item 7a, Face Page*)

\$ 2,502

CONSORTIUM/CONTRACTUAL COSTS

FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD

\$ 2,502

**BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD (from Form Page 4)	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: <i>Salary and fringe benefits. Applicant organization only.</i>	2,502				
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES					
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES					
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS (Sum = Item 8a, Face Page)	2,502				
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	2,502				
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD					\$ 2,502

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

The only direct costs applicable to this request are for the salary of the Project Intern for the duration of the Initial Budget Period. The Project Intern will be paid at the minimum wage rate defined by the State of Texas which is \$7.25/hour. The Project Intern will work a full 40 hours for the duration of the Initial Budget Period (8 weeks or 2 months).

CHECKLIST**TYPE OF APPLICATION** (Check all that apply.)

- ☐ NEW application. (This application is being submitted to the PHS for the first time.)
- ☐ RESUBMISSION of application number: _____
(This application replaces a prior unfunded version of a new, renewal, or revision application.)
- ☐ RENEWAL of grant number: _____
(This application is to extend a funded grant beyond its current project period.)
- ☒ REVISION to grant number: 1R01AI098775-01
(This application is for additional funds to supplement a currently funded grant.)
- ☐ CHANGE of program director/principal investigator.
Name of former program director/principal investigator: _____
- ☐ CHANGE of Grantee Institution. Name of former institution: _____
- ☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved: _____

INVENTIONS AND PATENTS (Renewal appl. only) ☒ No ☐ YesIf "Yes," ☐ Previously reported ☐ Not previously reported**1. PROGRAM INCOME** (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
N/A		

2. ASSURANCES/CERTIFICATIONS (See instructions.)

In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINISTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

- ☒ DHHS Agreement dated: 03/02/2010; 06/24/2010 ☐ No Facilities And Administrative Costs Requested.
- ☐ DHHS Agreement being negotiated with _____ Regional Office.
- ☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)

a. Initial budget period:	Amount of base \$	<u>2,502</u>	x Rate applied ☒	<u>57.3</u>	% = F&A costs	\$	<u>1,434</u>
b. 02 year	Amount of base \$		x Rate applied		% = F&A costs	\$	
c. 03 year	Amount of base \$		x Rate applied		% = F&A costs	\$	
d. 04 year	Amount of base \$		x Rate applied		% = F&A costs	\$	
e. 05 year	Amount of base \$		x Rate applied		% = F&A costs	\$	
TOTAL F&A Costs						\$	1,434

*Check appropriate box(es):

- ☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base (Explain)
- ☐ Off-site, other special rate, or more than one rate involved (Explain)

Explanation (Attach separate sheet, if necessary.):

4. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? ☐ Yes ☒ No

BIOGRAPHICAL SKETCH

NAME Peter Hotez MD PhD		POSITION TITLE Dean, National School of Tropical Medicine, Professor of Pediatrics, Molecular Virology & Microbiology, Baylor College of Medicine; President, Sabin Vaccine Institute	
eRA COMMONS USER NAME eRA Commons User Name			
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Yale University (magna cum laude)	BA	1976 - 1980	Molec. Biophysics
Rockefeller University	PhD	1980 - 1986	Biochemistry (Parasitol.)
Cornell University Medical College	MD	1980 - 1987	Medical Science
Harvard Medical School (Mass. Gen. Hosp)	Residency	1987 - 1989	Pediatrics
Yale University School of Medicine	Fellowship	1989 - 1991	Molecular Parasitology

A. Personal Statement.

Professor Peter Hotez is a laboratory and clinician investigator with a major interest in vaccines for neglected tropical diseases (NTDs) and emerging infections. He is an elected member of the Institute of Medicine and founding director of one of the first non-profit public private partnerships (PDP) for developing vaccines to combat NTDs. PDPs use industrial practices but develop products in the non-profit sector because they target diseases of the world's poorest people. Launched in 2000, the PDP of the Sabin Vaccine Institute filed its first Investigational New Drug (IND) application for a recombinant vaccine in 2004, with a second IND filed this year, both for hookworm infection. Today the Sabin PDP is also developing recombinant vaccines for major disease affecting developing countries, including hookworm, schistosomiasis, Chagas diseases and SARS. These vaccines are engineered in yeast and bacteria. Process development at the 10 liter fermentation scale, followed by protein purification and formulation studies are conducted at Sabin followed by technology transfer to a pilot scale manufacturer either in the U.S., Brazil, and Mexico, and then clinical testing in either the U.S. or developing countries. Thus, Sabin's PDP has acquired a track record of transitioning concepts and discoveries into actual vaccines for clinical testing. Sabin's PDP relocated to Baylor College of Medicine in, 2011 where it is known as Sabin Vaccine Institute and Texas Children's Center for Vaccine Development. He is also the founding Dean of the National School of Tropical Medicine at Baylor College of Medicine. Dr. Hotez is a recognized leader on vaccine development for tropical diseases and other infectious diseases.

B. Positions and Honors.

Positions and Employment

1991-92	Instructor, Pediatrics, Yale University
1992-95	Assistant Professor, Pediatrics/Epidemiology & Public Health, Yale University
1995-00	Associate Professor, Epidemiology & Public Health/Pediatrics, Yale University
2000-11	Professor and Chair, Department of Microbiology, Immunology and Tropical Medicine, The George Washington University
2006-11	Walter G. Ross Professor of Basic Science Research, The George Washington University
2008-11	Distinguished Research Professor, The George Washington University
2007-	President, Sabin Vaccine Institute
2007-	Editor-in-Chief, <i>PLoS Neglected Tropical Diseases</i>
2010-11	President, American Society of Tropical Medicine and Hygiene
2011-	Professor, Department of Pediatrics and Molecular Virology & Microbiology, and Head of Pediatric Tropical Medicine, Baylor College of Medicine

2011- Texas Children's Hospital Endowed Chair of Tropical Pediatrics
 2011- Dean, National School of Tropical Medicine at Baylor College of Medicine
 2013- Fellow in Disease and Poverty, James A. Baker III Institute, Rice University

Honors (after 2005)

2006 Leverhulme Medal, Liverpool School of Tropical Medicine
 2006 Ambassador, Paul G. Rogers Society for Global Health Research, ResearchAmerica!
 2007 Member of Advisory Board (Council), Fogarty International Center, National Institutes of Health
 2008 Science and Technical Advisory Group on Neglected Tropical Diseases, WHO
 2008 Elected Member, Institute of Medicine of the U.S. National Academies
 2009 Science and Technical Advisory Committee on Tropical Disease Research, WHO
 2011 Member, National Institutes of Health (NIH) Council of Councils
 2011 Elected Member, The Academy of Medicine, Engineering, and Science of Texas (TAMEST)
 2011 Fellow of the American Society of Tropical Medicine and Hygiene (FASTMH)
 2011 Abraham Horwitz Award for Excellence in Leadership in Inter-American Public Health, PAHO, WHO
 2012 Elected Member, The Association of American Physicians (AAP)
 2012 The Ralph D. Feigin, M.D. Award for Excellence, The Immunization Partnership
 2013 Vaccine Nation, Top 50 most influential vaccine personalities

C. Selected peer-reviewed publications (in chronological order). (Selected from 275)

1. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm Infection. *N Engl J Med* 2004; 351:799-807.
2. Asojo OA, Goud G, Dhar K, Loukas A, Zhan B, Deumic V, Liu S, Borgstahl GE, Hotez PJ. X-ray structure of Na-ASP-2, a pathogenesis-related-1 protein from the nematode parasite, *Necator americanus*, and a vaccine antigen for human hookworm infection. *J Mol Biol* 2005; 346:801-14.
3. Hotez PJ. Neglected Infections of Poverty in the United States of America. *PLoS Negl Trop Dis* 2008; 2:e256. PMID: PMC2430531.
4. Bethony JM, Simon G, Diemert DJ, Parenti D, Desrosiers A, Schuck S, Fujiwara R, Santiago H, Hotez PJ. Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 hookworm vaccine in unexposed adults. *Vaccine* 2008; 26: 2408-17.
5. Hotez PJ, Fenwick A, Savioli L, Molyneux DH. Rescuing the "bottom billion" through neglected tropical disease control. *Lancet* 2009; 373: 1570-4.
6. Hotez PJ. Peace through vaccine diplomacy. *Science* 2010; 327: 1301.
www.sciencemag.org/content/327/5971/1301.full?sid=5be8053a-cc9c-4381-a6e9-815c955ba849
7. Pearson MS, Pickering DA, Tribolet L, Cooper L, Mulvenna J, Oliveira LM, Bethony JM, Hotez PJ, Loukas A. Neutralizing antibodies to the hookworm hemoglobinase *Na-APR-1*: implications for a multivalent vaccine against hookworm infection and schistosomiasis. *J Infect Dis* 2010; 201:1561-9.
jid.oxfordjournals.org/content/201/10/1561.full
8. Zhan B, Perally S, Brophy PM, Xue J, Goud G, Liu S, Deumic V, de Oliveira LM, Bethony J, Bottazzi ME, Jiang D, Gillespie P, Xiao SH, Gupta R, Loukas A, Ranjit N, Lustigman S, Oksov Y, Hotez P. Molecular Cloning, Biochemical Characterization and Partial Protective Immunity of the Heme-binding Glutathione S-Transferases from the Human Hookworm *Necator americanus*. *Infect Immun* 2010; 78:1552-63. PMID: PMC2849424
9. Hotez PJ, Bethony JM, Diemert DJ, Pearson M, Loukas A. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat Rev Microbiol* 2010; 8: 814-26.
10. Hotez PJ, Mistry N, Rubinstein J, Sachs JD. Integrating neglected tropical diseases into AIDS, tuberculosis, and malaria control. *N Engl J Med* 2011; 364: 2086-9.
11. Hotez PJ. A handful of 'antipoverty' vaccines exist for neglected diseases, but the world's poorest billion people need more. *Health Aff (Millwood)* 2011; 30: 1080-7. PMID: 21653960.
12. Goud GN, Deumic V, Gupta R, et al. Expression, purification, and molecular analysis of the *Necator americanus* glutathione S-transferase 1 (Na-GST-1): a production process developed for a lead candidate recombinant hookworm vaccine antigen. *Protein Expr Purif* 2012; 83: 145-51.

13. Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, Jones K, Zhan B, Hotez PJ. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccines for severe acute respiratory syndrome. *Exp Rev Vaccines* 2012; 11: 1405. PMID: 23252385
14. Lee BY, Bacon KM, Bottazzi ME, Hotez PJ. The global economic burden of Chagas disease: a computational simulation model. *LANCET INFECTIOUS DISEASES* 2013; [Epub ahead of print].

D. Research Support

Ongoing Research Support

23386 Hotez (PI) 01/01/2011-12/31/2014

Sponsor: Dutch Government

Title: Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the *Na*-GST-1 and *Na*-APR-1 hookworm antigens in both adults and children.

1016395 Hotez (PI) 08/01/2011 - 07/31/2013

Sponsor: (b)(4)

Title: Human Hookworm Vaccine Initiative 3

Clinical Development and Evaluation of the *Na*-GST-1 and *Na*-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Hotez (PI) 04/20/2012 – 03/19/2014

(b)(4)

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

NIH R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

Sponsor: National Institutes of Health

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi Center Director 07/01/2012 – 12/31/2017

Sponsor: Department of Health and Human Services / Texas A&M Univ.

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development

Role: Instructor

Completed Research Support (selected support after 2009)

32472 Hotez (PI) 08/01/2006 – 12/31/2012

Sponsor: Sabin Vaccine Institute is conduit for The Bill and Melinda Gates Foundation

Title: Human Hookworm Vaccine Initiative 1

Human Hookworm Vaccine Initiative (HHVI): Clinical Development & Evaluation of the *Na*-ASP-2 Hookworm Vaccine

The goal of this study is to continue product development, including the manufacture of a second pilot lot, and to conduct a global health impact analysis of the human hookworm vaccine with the Sabin Vaccine institute

A/90577 Hotez (PI) 01/01/2011 – 12/31/2012

Sponsor: National Institutes of Health

Title: Product development of a membrane tetraspanin vaccine against schistosomiasis

The goal of this project is the development of a high-yield, low-cost process for producing and formulating a

recombinant Sm-TSP-2 schistosomiasis vaccine (10-liter scale)

RCA8608 Hotez (PI) 09/30/2009 - 09/30/2011

(b)(4) -PATH-MVI

Development of a Malaria transmission blocking Vaccine

To develop a feasibility of expression study for the expression and scale-up of the Anopheles-APN-1 target candidate antigen as a malaria transmission blocking vaccine. The goal of this study is to conduct the early feasibility of expression using both yeast and bacterial expression systems.

38988 Hotez (PI) 08/01/2006 - 07/31/2011

Human Hookworm Vaccine Initiative 2

Sabin Vaccine Institute-Human Hookworm Vaccine Initiative (HHVI): To develop and test the Na-APR-1 Hookworm Vaccine

The goal of this study is to conduct the process development, cGMP manufacture and testing, and clinical evaluation of APR-1 in order to develop a bivalent human hookworm vaccine with the Sabin Vaccine Institute

SUMMARY OF THE PROJECT SPECIFIC AIMS AND APPROACH

Funds are requested for a minority supplement to the parent grant AI-098775 on the development and manufacture of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV). The project will serve as a basis for engaging an under-represented minority high school student in an eight-week long mentored program of molecular biology and biochemistry research. The program will be offered in association with the Office of Diversity and Community Outreach at Baylor College of Medicine.

SPECIFIC AIMS. Briefly, the parent grant seeks to develop a recombinant vaccine against SARS, now classified by NIAID as a Category C Priority Pathogen, by advancing the vaccine through early stage expression and preclinical characterization, process development, formulation, stability, technology transfer and cGMP manufacture. The Actual Specific Aims of the parent grant are as follows:

Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. We will evaluate the expression of the recombinant RBD (rRBD) in bacteria and yeast expression systems and select one of them for expression of rRBD protein for subsequent studies based on yields, purity, stability, antigenicity, functionality, immunogenicity, and efficacy (for inducing neutralizing antibody responses and protection against SARS-CoV challenge) of the rRBD protein when formulated in alum. We will use the rRBD protein from the selected expression system for optimization of immunization regimens, and assess the ability of rRBD protein formulated with alum based adjuvants and/or GLA, a TL R4 antagonist adjuvant, to induce cross-neutralizing antibody response, cross-protection and long-term immune responses and protection in mouse models using the optimized immunization regimen. (Timeline Year 1-3)

Specific Aim 2: Process development, characterization, formulation and stability profiling. In parallel to Aim 1, a scalable and reproducible fermentation process for rRBD (10 liter scale) and a purification process using chromatographic technologies will be developed. Reproducibility will be confirmed and specific product quality assays will be developed and used to characterize the recombinant vaccine protein. Vaccine buffer formulations will be developed and characterized using an innovative approach, combining analytical/biochemical tools with biophysical assays to test different excipients and stabilizers and establish an optimal stability profile. The stabilized protein will be formulated with alum and/or GLA. The binding and effect on the structure stability will be examined. Immunogenicity and efficacy of the vaccine formulations will be evaluated in parallel as described in Aim 1. The assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. (Timeline Year 2-4)

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. The cell bank production, production processes and formulation technology for the selected rRBD-based vaccine will be transferred to Walter Reed Army Institute of Research (WRAIR) pilot facility for 60-L scale GMP manufacture, formulation and fill and finish. The clinical lots will be released by BCM-Sabin and following a pre-IND meeting with the U.S. FDA, GLP toxicology will be initiated at Frontier Biosciences, a Maryland-based contractor. BCM-Sabin will prepare and submit an IND in preparation for the initiation of the clinical development plan. (Timeline Year 4-5)

With the exception of the final cGMP manufacture and preclinical testing for immunogenicity and efficacy using a mouse challenge model all of the work is conducted at the Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine (BCM-Sabin).

APPROACH. The strengths of our approach rely on an extensive evidence base of preliminary data collected over the last seven years by our collaborators on the grant at the New York Blood Center (NYBC), which point to the RBD protein as a lead candidate vaccine antigen, together with an eleven-year track record of product development and testing for recombinant vaccines by the Sabin Vaccine Institute Product Development Partnership now based at Baylor College of Medicine (BCM-Sabin). Additionally, BCM-Sabin has had previous success in tech transferring processes for recombinant protein vaccines to Walter Reed Army Institute of Research, our cGMP contractor (CMO) of choice. Further, the group has demonstrated the feasibility of developing rRBD-based subunit vaccines. Specifically, our collaborators at NYBC have shown that mammalian cell-expressed rRBD fused induces high titer of RBD-specific neutralizing antibodies in vaccinated animals and long-term (over a year) immune responses and protection against subsequent SARS-CoV challenge, while using the pseudotyped viruses expressing S protein originated from Tor2, GD03, or SZ3, the representative strains of human 2002–2003 & 2003–2004 SARS-CoV and palm civet SARS-CoV, respectively, we also found that mouse and rabbit antibodies raised against rRBD derived from either one of the aforementioned strains of SARS-CoV and the palm civet SARS-CoV cross-neutralize one another, suggesting their protective efficacy against challenge with heterogeneous viruses. Immunization of mice with rRBD derived from various expressing systems, including mammalian cells (293T and CHO), insect sf9 cells, and *E. coli* is capable of producing high-levels of RBD-specific neutralizing antibody and potent T cell responses against both pseudotyped and live SARS-CoV. Importantly, these rRBD proteins appear to maintain intact conformation and authentic antigenicity, reacting with the RBD-specific and conformation-dependent mAbs with neutralizing activity. These rRBD proteins elicited immunity that protected all vaccinated mice from SARS-CoV challenge. These preliminary data and expertise by NYBC is now being paired with the track record at BCM-Sabin/ WRAIR for transitioning a recombinant protein-based vaccine through process development and formulation, technology transfer for cGMP manufacture, lot release and stability testing, and GLP toxicology testing. All steps needed in order to compile a regulatory file application (IND) in preparation for a submission to the FDA.

The Major Deliverables

1. Development of a scalable, reproducible process for expression of the rRBD protein and formulation of the RBD-based vaccine with high quality and stability.
2. Establishment of an immunization regimen, including the optimized antigen dose, vaccination schedule and route and adjuvant formulation, for evaluation of the immunogenicity and efficacy of the vaccine.
3. Successful pilot cGMP manufacture of a subunit SARS vaccine comprised of rRBD protein and an adjuvant formulation.
4. Completion of the GLP toxicology testing with acceptable safety profile.
5. IND preparation and submission with U.S. FDA.

The parent grant was written and submitted in response to an RFA in bio defense - RFA-AI-11-014 Partnerships for Biodefense (R01) - BCM-Sabin is applying its proven product development partnership approach and strong peer-reviewed publication track record in research and development of parasitic recombinant protein vaccines to accelerate the development of a recombinant RBD-based vaccine to prevent SARS-CoV infection. To ensure success, BCM-Sabin provides program management throughout the project timeline and leverage the collaborations with the research and industry partners to augment the expertise for anti-SARS-CoV vaccine testing. The strengths of this proposal rely on the supporting proof of concept data that identifies the RBD protein as a lead candidate vaccine antigen selected for further process development, characterization and preclinical evaluation. Following feasibility of expression in both bacterial and yeast expression systems, the recombinant proteins are assessed at BCM-Sabin for scalability, yield, quality and stability. Antigenicity, functionality, immunogenicity and potency of the vaccine formulations will be developed and evaluated at NYBC and additional collaborators at University of Texas Medical Branch Galveston (UTMB). Furthermore, rRBD protein from the selected expression system will be compared at NYBC and UTMB for its ability to induce neutralizing antibodies and protection in laboratory animals against challenge infections with multiple strains of the SARS-CoV using different adjuvant platforms; alum (either Alhydrogel® or aluminum phosphate) and/or GLA, a TLR4 antagonist adjuvant. Once the most efficacious expression system is selected, BCM-Sabin will perform scale-up process development (PD) at the 10 liter fermentation scale followed by protein purification under detailed documentation. Reproducibility will be confirmed and specific product quality control assays will be developed in collaboration with NYBC and used to characterize rRBD. In

addition, vaccine buffer formulations will be developed and characterized, and excipients and stabilizers will be evaluated. The stabilized molecule will then be formulated with alum and/or GLA and their effect on the structure stability will also be examined. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. The developed process will then be transferred to WRAIR that successfully manufactured two hookworm vaccines with BCM-Sabin. After pilot lot material is produced and released and following a pre-IND meeting with the U.S. FDA, toxicology testing will commence and an IND application will be prepared. Thus a key strength is clear-cut project deliverables (see adjoining box).

The proposed project is ideal for mentoring a under-represented highschool student on fundamental techniques in molecular biology and biochemistry and providing a broad educational overview on the key steps in developing a vaccine to prevent a major public health threat.

PLAN/TIMELINE FOR RESEARCH AND CAREER DEVELOPMENT (INCLUDING MENTORSHIP ACTIVITIES)

We have planned for an 8 week period of research. During this period of research, the highschool student will meet with our research team regularly to observe research meetings, and also contribute to the meetings once they start collecting data. The student will be mentored through the entire research period and trained in various general aspects of research. Each week the student will have a one-on-one mentorship meeting where they can give feedback on their activities for the week and point out any areas that they may not understand, or may need additional training with.

The detailed RESEARCH AND MENTORSHIP PLAN is described below with a set of targets for each week:

Week 1:

- Laboratory orientation and rules
- Overview of Ethical guidelines in research – explanation of human subjects and animal protections
- Introduction to molecular biology and biochemistry, with emphasis on recombinant DNA technologies
- Overview of Professionalism
- Training in basic knowledge of nucleic acids, proteins, and respiratory viruses
- Attendance of laboratory meeting (group)
- Review of the research strategy and timelines (with Principal Investigator)

Weeks 2-6

Training and orientation on basic techniques in molecular biology and biochemistry

- How to prepare buffers and reagents
- How to prepare and run agarose gels and visualize and quantitate DNA
- How to use restriction endonucleases
- How to prepare plasmids and transfect yeast and bacteria
- How to induce protein expression and analyze recombinant proteins on SDS-PAGE

Week 2:

- Record manipulations and observations – Preparation of buffers and reagents; agarose gels and visualizing and quantitating DNA
- Attendance of laboratory meeting (group)
- Overview of the National Institutes of Health
- Mentorship meeting (one-on-one)

Week 3:

- Record manipulations and observations – use of restriction endonucleases and ligases
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training on how to use empirical data in preparing a research project
- Training in how to find empirical data and how to organize it

Week 4:

- Record manipulations and observations –preparing plasmids and yeast and bacterial transfection
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training in scientific writing and on how to write an abstract

Week 5:

- Record manipulations and observations –preparing plasmids and yeast and bacterial transfections
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training in the history and function of the IRB

Week 6:

- Record manipulations and observations – protein expression and analysis of recombinant proteins on SDS PAGE
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training in oral presentation skills

Week 7:

- Record manipulations and observations – protein expression and analysis of recombinant protein on SDS PAGE.
- Evaluation of experiments and data collection
- Introduction to creating a research poster
- Attendance of laboratory meeting (group)
- Produce a draft presentation of research findings with the assistance of mentor (one-on-one)

Week 8:

- Meetings with PI and laboratory staff to review results
- Finalize draft of research findings
- Presentation of findings at laboratory meeting
- Provide feedback on how to improve laboratory experiences
- Reflect on the summer research experience

Evidence of mentoring experience for the Principal Investigator:

Peter Hotez MD PhD - the Principal Investigator - is an elected member of the Institute of Medicine with more than 25 years of experience leading laboratory investigations on neglected and infectious diseases. Over the last decade he has led a non-profit product development partnership for the development, manufacture, and clinical testing of recombinant protein vaccines to combat neglected tropical diseases, and was recently named one of the fifty most influential people in vaccines. Dr. Hotez has supervised or co-supervised at least a dozen postdoctoral fellows and a dozen research faculty, in addition to three PhD candidates for their doctoral dissertations (including an MD PhD student now in training). He has also supervised the thesis work of numerous students for their master degree in public health (MPH) and related degrees. He is widely sought out by medical and graduate students, as well as undergraduates for career advice.

For this mentored program much of the direct training at the laboratory bench will be led by senior laboratory staff members and research-intensive faculty working in the larger laboratory group of Dr. Hotez. Similar arrangements have led to numerous successful mentoring experiences for other high school students and undergraduates. Last summer, two high school students received laboratory instruction and training in the Hotez laboratory.

Description of how the research and career development experiences will expand and foster the research capabilities of the candidate:

The described course of summer study will introduce the student to the basic principals of biomedical scientific research. The student will understand the planning process and the approaches that are used to develop a project, analyze the data, and in the preparation of data for presentation. The candidate/student will gain a basic understanding of molecular biology and biochemistry of macromolecules (nucleic acids and proteins) and how these approaches contribute to our understanding of the mechanisms that allow respiratory viruses to invade host tissues and how vaccines may be used to combat them. The student will gain experience in laboratory techniques, procedures, biosafety and ethics.

Under the guidance of experienced researcher the student will learn fundamental techniques in molecular biology and biochemistry including buffer and reagent preparation, agarose gels and DNA visualization and quantitation, use and application of restriction endonucleases, preparation of plasmids and transfection of yeast and bacteria, fusion protein induction and expression, and analysis of recombinant proteins on SD S-PAGE. These studies will be conducted in the larger context and environment of the steps needed to develop and test recombinant protein vaccines for important infectious agents and biodefense threats including SARS. Thus we hope to stimulate the imagination of the trainee about the role he/she could one day have in the important area of public health emergency preparedness.

Equally important, this student will observe the scientific process unfold in a laboratory setting. We anticipate that the student will become engaged in the practice and discipline of academic research and, since this is biomedical research, come to understand and appreciate the role of research in the practice of medicine. The overall goal is for this high school student is to see the challenges and opportunities that exist in arenas of higher education. One of the goals is to instill a sense of awe and excitement in the student as to all of the possibilities available to them in their future. It is our objective to provide this student with world-class training and experience in an 8 week defined course of study.

In conjunction with the Office of Diversity and Community Outreach at Baylor College of Medicine, the student will present a research poster at the annual student research symposium that this office sponsors each year in January.

When I was young, I had always wanted to become a doctor. From watching the Discovery Health channel to playing with the instruments at the doctor's office, I was just fascinated with the profession. I loved the idea of becoming a doctor, but I loved working with science even more. My mother is a middle school life science teacher and I would always help her set up for laboratory experiments and even perform a couple with my mother. Honestly, it just seemed fun at the time. That innocent fun soon transformed into a passion to gain understanding of our natural world. The fact that science is ever-changing and never static is what excites my curiosity. Every day, new things are being discovered, theories being tested, and cures are being found, and I want to someday be a part of that amazing process.

Currently, I am involved in the Science National Honor Society and S.T.E.M Club at my high school, and participated in science fair completions at the district and regional levels. One of my experiments, titled "CSI: Daphnia," explored how the contamination of aquatic environments from ordinary pollutants (common runoff pollutants) of varying concentrations can harm aquatic life. I used the *Daphnia pulex* in my research because of their transparency and visible heart beatings and palpitations, if present. Conducting this experiment gave me a small dose of what biomedical research is like, and I absolutely loved it. I remember telling my science teacher Dr. Dilmaliwat that I felt like a scientist as I preformed the experiment. She smiled and told me that it was because I *was* a scientist. I was mind blown. It did not occur to me that what I was doing was exactly what researchers in universities and professionals are doing. Confucius once said "choose a job you love, and you will never have to work a day in your life." His words hit me hard, because I have done tedious and exhausting tasks at Sears but only simply exercised my passion with conducting experiments and research in the great name of science.

I had the honor and privilege of meeting Lisa Jackson, administrator of the Environmental Protection Agency, during a seminar in the summer of 2012. Mrs. Jackson described her journey through college and graduate school at the Princeton University as a winnable challenge despite not only being a person on ethnic minority but also as a woman in a male dominated field. I was in awe throughout the entire presentation. I can honestly say that that day I was (and still am) inspired to keep pushing forward in the face of adversity. As the first person on African American descent to serve as Administrator of EPA, Lisa Jackson made a name for all of her hard work and dedication – something I longed for myself one day.

Participating in SMS Summer Intern Program would be an invaluable experience for me, considering my academic interests and career goals. Not only that, participation in this internship will help me grow as a young college bound researcher on the path to practice medicine as a profession. If given the opportunity of this internship, I will not disappoint. I will go above and beyond and make great strides to becoming the best that I can be, for my mother, my community, my country, and for the sake of science.

Sincerely,

Frederick A. Nelson

Frederick Nelson

Personal Info

WORK EXPERIENCE:

Alief Early College School Newspaper: *Knightly News*, Chief Editor

- Manage and supervise staff
- Publish and distribute final copies of the newspaper

Sears Outlet: Sales Associate (8/25- Present)

- Sell new, used, discontinued, scratch and dent appliances

HONORS AND AWARDS:

Science Fair (District) 1ST Place: 10th grade

Science Fair (District) 4th Place: 11th grade

Microsoft Office 2007 Word Certified

AP Scholar Award (College Board)

Houston Community College Dean's List: 11th grade

Honor Roll: 10th, 11th, 12th grade

ACTIVITIES:

School Newspaper: 11th grade

Student Council: 9th, 10th, 11th, 12th grades

National Science Honor Society: 11th, 12th grades

National Honor Society: 11th, 12th grade

Texas Governor's School of Leadership in the Humanities: 10th, 11th grades

National Society of Collegiate Scholars: 12th grade

Class Officers: 10th, 12th grades

Saturday Morning Science at the Baylor College of Medicine: 12th grade

COMMUNITY SERVICE:

PAIR (Partnership for the Advancement & Immersion of Refugees): 11th grade

- Mentored and tutored refugee youth at Fondren Middle School (Fall 2011, Spring 2012)

Community Service Club: 10th, 11th grades

- Participated in various events and service projects in the Alief community
 - Served as a vendor with Chinese New Year Festival, 1/27/12, 9 Hours
 - Helped to clean ACC Park, 2/26/11, 4 Hours
 - Assisted with clean up at Boone park, 2/12/11, 6 Hours
 - Assisted in tree planting at Hackberry Park, 1/29/11, 8 Hours
- Ushered at the Hobby Center, 12/15/12, 6 Hours



To whom it may concern:

(b)(6) (Name of Student), a student from Alief Early College High School

(Insert name of school), located at 2811 Hayes Rd. (school address), would like to participate in the summer research experience offered to them through the Saturday Morning Science program at Baylor College of Medicine for the summer of 2013. I understand that said student will be working in a research lab under the direction of a Principal Investigator at Baylor College of Medicine who is operating under an active grant funded through the National Institutes of Health.

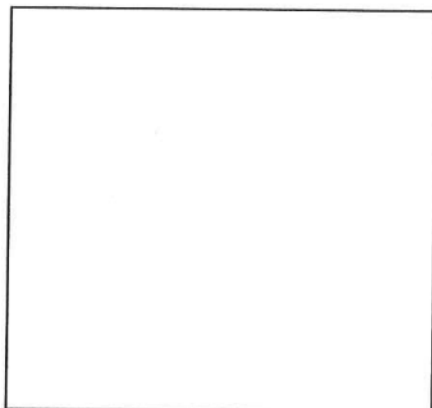
I approve of (b)(6)'s (name of student) participation in this research program, and I attest that their participation will not detract from or interfere with the student's course of studies.

Name of school official completing document: Terri Guidry

Role of school official: Counselor Phone #: 281-988-3010

Signature of school official: (b)(6) Date: 2/28/13

Please affix school stamp or seal in the designated space below:



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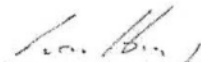
Withheld pursuant to exemption

Personal Info

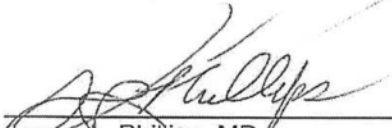
of the Freedom of Information and Privacy Act

Statement of Eligibility from the Investigator

Frederick Nelson PII [redacted] is a senior at Alief Early College High School in Houston, Texas, and is a U.S. citizen PII [redacted]. We hope that the early exposure of this candidate to this area of scientific research and the experience he will gain by participating in a laboratory study will further influence his decision to pursue a career in the healthcare field, therefore impacting the national scientific workforce. This experience will also be beneficial to Frederick as he begins to make decisions about the pursuit of his undergraduate studies. Additionally, this experience will provide a better understanding of the relationship between research studies and the delivery of healthcare, as well as basic research methods. His presence here at Baylor in the capacity of project intern for the summer will further enhance our institution's goal of diversity, and its goal to have an impact in reducing the racial and ethnic disparity in the scientific and healthcare workforce. Frederick Nelson has not received any previous or current PHS support.



Peter Hotez, MD, PhD
Principal Investigator
Dean of School of Tropical Medicine
Baylor College of Medicine



James L. Phillips, MD
Senior Associate Dean
Office of Diversity and Community Outreach
Baylor College of Medicine



Leanne B. Scott, PhD
Director, Sponsored Programs
Baylor College of Medicine



RESEARCH

Department of Health and Human Services

National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Notice of Award

Issue Date: 06/16/2014



Grant Number: 3R01AI098775-03S1

FAIN: R01AI098775

Principal Investigator(s):

Maria Elena Bottazzi

PETER J HOTEZ (contact)

SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Leanne Brooks Scott

Business Official

One Baylor Plaza, BCM320A

Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Budget Period: 06/16/2014 – 04/30/2015

Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$3,936 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Michael W. Fato
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 3R01AI098775-03S1**Award Calculation (U.S. Dollars)**

Salaries and Wages	\$2,320
Fringe Benefits	\$182
Federal Direct Costs	\$2,502
Federal F&A Costs	\$1,434
Approved Budget	\$3,936
Federal Share	\$3,936
TOTAL FEDERAL AWARD AMOUNT	\$3,936
AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$3,936

SUMMARY TOTAL FEDERAL AWARD AMOUNT YEAR (3)	
GRANT NUMBER	TOTAL FEDERAL AWARD AMOUNT
3R01AI098775-03S1	\$3,936
5R01AI098775-03	\$1,134,359
TOTAL	\$1,138,295

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
3	\$3,936	\$1,138,295
4	\$0	\$1,165,726
5	\$0	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number: 93.855
EIN: 1741613878A1
Document Number: RAI098775A

PMS Account Type: G (Pooled)
Fiscal Year: 2014

IC	CAN	2014
AI	8012165	\$3,936

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC: M51C B / OC: 414C / Released: 06/10/2014
Award Processed: 05/08/2014 01:52:21 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 3R01AI098775-03S1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 3R01AI098775-03S1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Award.

- b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm> for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI098775. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 3R01AI098775-03S1

This award provides support under the Research Supplements to Promote Diversity in Health-Related Research Program for Ebe Ewere at the Baylor College of Medicine beginning 06/09/2014. These funds (\$2,502 Direct Costs and \$1,434 Facilities and Administrative Costs) are restricted for the above purpose only and may not be transferred to any other individual. Funds awarded are available for carryover for awards given carryover authority as reflected in section III of this award notice. However, the funds remain restricted for the individual and the purpose for which the supplement is awarded.

In future years a separate progress report for the Research Supplement to Promote Diversity in Health-Related Research is required as part of the progress report of the parent grant.

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH

awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Jason A. Lundgren
Email: lundgrenj@mail.nih.gov **Phone:** 301-594-6355 **Fax:** 301 493 0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone:** (301)-402-3947

SPREADSHEET SUMMARY

GRANT NUMBER: 3R01AI098775-03S1

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	Year 3	Year 4	Year 5
Salaries and Wages	\$2,320		
Fringe Benefits	\$182		
TOTAL FEDERAL DC	\$2,502		
TOTAL FEDERAL F&A	\$1,434		
TOTAL COST	\$3,936	\$0	\$0

Facilities and Administrative Costs	Year 3	Year 4	Year 5
F&A Cost Rate 1	57.3%		
F&A Cost Base 1	\$2,502		
F&A Costs 1	\$1,434		

**Research Supplement to Promote Diversity
in Health-Related Research**

<http://grants.nih.gov/grants/guide/pa-files/PA-12-149.html>

Principal Investigator: Peter Hotez, MD, Ph.D.

Diversity Investigator: Ebe Ewere (High School Student)

Grant #: R01 AI 98775

Program Code: M51C B

Program Official: Erik Stemmy

Department of Health and Human Services Public Health Services Grant Application <i>Do not exceed character length restrictions indicated.</i>		LEAVE BLANK—FOR PHS USE ONLY. <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Type</td> <td style="width: 33%;">Activity</td> <td style="width: 34%;">Number</td> </tr> <tr> <td colspan="2">Review Group</td> <td>Formerly</td> </tr> <tr> <td colspan="2">Council/Board (Month, Year)</td> <td>Date Received</td> </tr> </table>		Type	Activity	Number	Review Group		Formerly	Council/Board (Month, Year)		Date Received
Type	Activity	Number										
Review Group		Formerly										
Council/Board (Month, Year)		Date Received										
1. TITLE OF PROJECT (Do not exceed 81 characters, including spaces and punctuation.) RBD RECOMBINANT PROTEIN-BASED SARS VACCINE FOR BIODEFENSE - 5R01AI098775-03												
2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES (If "Yes," state number and title) Number: PA-12-149 Title: Research Supplements to Promote Diversity in Health Related Research												
3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR												
3a. NAME (Last, first, middle) Hotez, Peter		3b. DEGREE(S) MD, PhD										
		3h. eRA Commons User Name eRA Commons User Name										
3c. POSITION TITLE Professor		3d. MAILING ADDRESS (Street, city, state, zip code) One Baylor Plaza, BCM 113 Houston, TX 77030-3411 E-MAIL ADDRESS: hotez@bcm.edu										
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Pediatrics, Tropical Medicine												
3f. MAJOR SUBDIVISION College of Medicine												
3g. TELEPHONE AND FAX (Area code, number and extension) TEL: 713-798-1199 FAX: 713-798-2299												
4. HUMAN SUBJECTS RESEARCH ☒ No ☐ Yes		4a. Research Exempt If "Yes," Exemption No. ☐ No ☐ Yes										
4b. Federal-Wide Assurance No.		4c. Clinical Trial ☐ No ☐ Yes										
		4d. NIH-defined Phase III Clinical Trial ☐ No ☐ Yes										
5. VERTEBRATE ANIMALS ☒ No ☐ Yes		5a. Animal Welfare Assurance No										
6. DATES OF PROPOSED PERIOD OF SUPPORT (month, day, year—MM/DD/YY) From 06/09/14 Through 08/01/14		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD 7a. Direct Costs (\$) \$2,502										
		7b. Total Costs (\$) \$3,936										
		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT 8a. Direct Costs (\$) \$2,502										
		8b. Total Costs (\$) \$3,936										
9. APPLICANT ORGANIZATION Name Baylor College of Medicine Address One Baylor Plaza Houston, TX 77030-3411		10. TYPE OF ORGANIZATION Public: → ☐ Federal ☐ State ☐ Local Private: → ☒ Private Nonprofit For-profit: → ☐ General ☐ Small Business ☐ Woman-owned ☐ Socially and Economically Disadvantaged										
		11. ENTITY IDENTIFICATION NUMBER 1741613878A1 DUNS NO.051113330 Cong. District TX-009										
12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE Name Leanne B. Scott, Ph.D. Title Director, Sponsored Programs Address Baylor College of Medicine One Baylor Plaza, BCM 310 Houston, TX 77030-3411 Tel: 713-798-1297 FAX: 713-798-6990 E-Mail: spo@bcm.edu		13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION Name Leanne B. Scott, Ph.D. Title Director, Sponsored Programs Address Baylor College of Medicine One Baylor Plaza, BCM 310 Houston, TX 77030-3411 Tel: 713-798-1297 FAX: 713-798-6990 E-Mail: spo@bcm.edu										
14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.		SIGNATURE OF OFFICIAL NAMED IN 13. (In ink. "Per" signature not acceptable.) 										
		DATE 4-30-14										

PROJECT SUMMARY (See instructions):

Funds are requested for a minority supplement to the parent grant AI-098775 on the development and manufacture of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV). The project will serve as a basis for engaging an under-represented minority high school student in an eight-week long mentored program of molecular biology and biochemistry research. The program will be offered in association with the Office of Diversity and Community Outreach at Baylor College of Medicine.

Briefly, the parent grant seeks to develop a recombinant vaccine against SARS, now classified by NIAID as a Category C Priority Pathogen, by advancing the vaccine through early stage expression and preclinical characterization, process development, formulation, stability, technology transfer and cGMP manufacture.

The Actual Specific Aims of the parent grant are as follows:

Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate.

Specific Aim 2: Process development, characterization, formulation and stability profiling. In parallel to Aim 1, a scalable and reproducible fermentation process for rRBD (10 liter scale) and a purification process using chromatographic technologies will be developed.

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation.

With the exception of the final cGMP manufacture and preclinical testing for efficacy using a mouse challenge model all of the work is conducted at the Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development of Baylor College of Medicine.

RELEVANCE (See instructions):

Under the guidance of an experienced researcher the minority student will learn fundamental techniques in molecular biology and biochemistry. These studies will be conducted in the larger context and environment of the steps needed to develop and test recombinant protein vaccines for important infectious agents and biodefense threats including SARS. Thus we hope to stimulate the imagination of the trainee about the role he/she could one day have in the important area of public health emergency preparedness.

PROJECT/PERFORMANCE SITE(S) (if additional space is needed, use Project/Performance Site Format Page)

Project/Performance Site Primary Location

Organizational Name: Baylor College of Medicine

DUNS: 051113330

Street 1: One Baylor Plaza

Street 2:

City: Houston

County: Harris

State: TX

Province:

Country: United States of America

Zip/Postal Code: 77030

Project/Performance Site Congressional Districts: TX-009

Additional Project/Performance Site Location

Organizational Name: Texas Childrens' Hospital

DUNS: 074615394

Street 1: 1102 Bates Ave

Street 2: Ste 550

City: Houston

County: Harris

State: TX

Province:

Country: United States of America

Zip/Postal Code: 77030

Project/Performance Site Congressional Districts: TX-009

Program Director/Principal Investigator (Last, First, Middle): **Hotez, Peter**

SENIOR/KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Program Director(s)/Principal Investigator(s). List all other senior/key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Peter Hotez, MD, PhD	eRA Commons User Name	BCM	PI
Oluwatoyin Asojo, PhD		BCM	Co-I

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project
James L. Phillips, MD	BCM	Diversity&Community Outreach

Human Embryonic Stem Cells ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: <http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp>. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

**DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY**FROM
06/09/2014THROUGH
08/01/2014List PERSONNEL (*Applicant organization only*)

Use Cal, Acad, or Summer to Enter Months Devoted to Project

Enter Dollar Amounts Requested (*omit cents*) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnth	Acad. Mnth	Summer Mnth	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Peter Hotez, MD, PhD	PD/PI	0						0
Ebe Ewere	Project Intern	EFFORT			Institutional Base Salary	2,320	182	2,502
Oluwatoyin Asojo, PhD	Co-I	0						
SUBTOTALS →						2,320	182	2502

CONSULTANT COSTS 0

EQUIPMENT (*Itemize*) 0SUPPLIES (*Itemize by category*) 0

TRAVEL 0

INPATIENT CARE COSTS 0

OUTPATIENT CARE COSTS 0

ALTERATIONS AND RENOVATIONS (*Itemize by category*) 0OTHER EXPENSES (*Itemize by category*) 0

CONSORTIUM/CONTRACTUAL COSTS DIRECT COSTS 0

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (*Item 7a, Face Page*) \$ 2,502

CONSORTIUM/CONTRACTUAL COSTS FACILITIES AND ADMINISTRATIVE COSTS 0

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD \$ 2,502

**BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD (from Form Page 4)	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: <i>Salary and fringe benefits. Applicant organization only.</i>	2,502				
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES					
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES					
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS (Sum = Item 8a, Face Page)	2,502				
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	2,502				
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD					\$ 2,502

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

The only direct costs applicable to this request are for the salary of the Project Intern for the duration of the Initial Budget Period. The Project Intern will be paid at the minimum wage rate defined by the State of Texas which is \$7.25/hour. The Project Intern will work a full 40 hours for the duration of the Initial Budget Period (8 weeks or 2 months).

CHECKLIST**TYPE OF APPLICATION** (Check all that apply.)

- ☐ NEW application. (This application is being submitted to the PHS for the first time.)
- ☐ RESUBMISSION of application number: _____
(This application replaces a prior unfunded version of a new, renewal, or revision application.)
- ☐ RENEWAL of grant number: _____
(This application is to extend a funded grant beyond its current project period.)
- ☒ REVISION to grant number: 5R01AI098775-03
(This application is for additional funds to supplement a currently funded grant.)
- ☐ CHANGE of program director/principal investigator.
Name of former program director/principal investigator: _____
- ☐ CHANGE of Grantee Institution. Name of former institution: _____
- ☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved: _____

INVENTIONS AND PATENTS (Renewal appl. only) ☐ No ☐ YesIf "Yes," ☐ Previously reported ☐ Not previously reported**1. PROGRAM INCOME** (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS (See instructions.)

In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINISTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

- ☒ DHHS Agreement dated: 03/02/2010; 06/24/2010 ☐ No Facilities And Administrative Costs Requested.
- ☐ DHHS Agreement being negotiated with _____ Regional Office.
- ☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)

a. Initial budget period:	Amount of base \$	<u>2,502</u>	x Rate applied	<u>57.3</u>	% = F&A costs	\$	<u>1,434</u>
b. 02 year	Amount of base \$	_____	x Rate applied	_____	% = F&A costs	\$	_____
c. 03 year	Amount of base \$	_____	x Rate applied	_____	% = F&A costs	\$	_____
d. 04 year	Amount of base \$	_____	x Rate applied	_____	% = F&A costs	\$	_____
e. 05 year	Amount of base \$	_____	x Rate applied	_____	% = F&A costs	\$	_____
TOTAL F&A Costs						\$	<u>1,434</u>

*Check appropriate box(es):

- ☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base (Explain)
- ☒ Off-site, other special rate, or more than one rate involved (Explain)

Explanation (Attach separate sheet, if necessary.):

Rate: 57.3% = BCM off campus rate 27.1% + TCH affiliate rate 30.2%

4. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? ☐ Yes ☒ No

BIOGRAPHICAL SKETCH

NAME Peter Hotez MD PhD		POSITION TITLE Dean, National School of Tropical Medicine, Professor of Pediatrics, Molecular Virology & Microbiology, Baylor College of Medicine; President, Sabin Vaccine Institute	
eRA COMMONS USER NAME eRA Commons User Name			
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Yale University (magna cum laude)	BA	1976 - 1980	Molec. Biophysics
Rockefeller University	PhD	1980 - 1986	Biochemistry (Parasitol.)
Cornell University Medical College	MD	1980 - 1987	Medical Science
Harvard Medical School (Mass. Gen. Hosp)	Residency	1987 - 1989	Pediatrics
Yale University School of Medicine	Fellowship	1989 - 1991	Molecular Parasitology

A. Personal Statement.

Professor Peter Hotez is a laboratory and clinician investigator with a major interest in vaccines for neglected tropical diseases (NTDs) and emerging infections. He is an elected member of the Institute of Medicine and founding director of one of the first non-profit public private partnerships (PDP) for developing vaccines to combat NTDs. PDPs use industrial practices but develop products in the non-profit sector because they target diseases of the world's poorest people. Launched in 2000, the PDP of the Sabin Vaccine Institute filed its first Investigational New Drug (IND) application for a recombinant vaccine in 2004, with a second IND filed this year, both for hookworm infection. Today the Sabin PDP is also developing recombinant vaccines for major disease affecting developing countries, including hookworm, schistosomiasis, Chagas diseases and SARS. These vaccines are engineered in yeast and bacteria. Process development at the 10 liter fermentation scale, followed by protein purification and formulation studies are conducted at Sabin followed by technology transfer to a pilot scale manufacturer either in the U.S., Brazil, and Mexico, and then clinical testing in either the U.S. or developing countries. Thus, Sabin's PDP has acquired a track record of transitioning concepts and discoveries into actual vaccines for clinical testing. Sabin's PDP relocated to Baylor College of Medicine in, 2011 where it is known as Sabin Vaccine Institute and Texas Children's Center for Vaccine Development. He is also the founding Dean of the National School of Tropical Medicine at Baylor College of Medicine. Dr. Hotez is a recognized leader on vaccine development for tropical diseases and other infectious diseases.

B. Positions and Honors.

Positions and Employment

1991-92 Instructor, Pediatrics, Yale University
 1992-95 Assistant Professor, Pediatrics/Epidemiology & Public Health, Yale University
 1995-00 Associate Professor, Epidemiology & Public Health/Pediatrics, Yale University
 2000-11 Professor and Chair, Department of Microbiology, Immunology and Tropical Medicine, The George Washington University
 2006-11 Walter G. Ross Professor of Basic Science Research, The George Washington University
 2008-11 Distinguished Research Professor, The George Washington University
 2007- President, Sabin Vaccine Institute
 2007- Editor-in-Chief, *PLoS Neglected Tropical Diseases*
 2010-11 President, American Society of Tropical Medicine and Hygiene
 2011- Professor, Department of Pediatrics and Molecular Virology & Microbiology, and Head of Pediatric Tropical Medicine, Baylor College of Medicine
 2011- Texas Children's Hospital Endowed Chair of Tropical Pediatrics
 2011- Dean, National School of Tropical Medicine at Baylor College of Medicine

2013- Fellow in Disease and Poverty, James A. Baker III Institute, Rice University

Honors (after 2005)

- 2006 Leverhulme Medal, Liverpool School of Tropical Medicine
- 2006 Ambassador, Paul G. Rogers Society for Global Health Research, ResearchAmerica!
- 2007 Member of Advisory Board (Council), Fogarty International Center, National Institutes of Health
- 2008 Science and Technical Advisory Group on Neglected Tropical Diseases, WHO
- 2008 Elected Member, Institute of Medicine of the U.S. National Academies
- 2009 Science and Technical Advisory Committee on Tropical Disease Research, WHO
- 2011 Member, National Institutes of Health (NIH) Council of Councils
- 2011 Elected Member, The Academy of Medicine, Engineering, and Science of Texas (TAMEST)
- 2011 Fellow of the American Society of Tropical Medicine and Hygiene (FASTMH)
- 2011 Abraham Horwitz Award for Excellence in Leadership in Inter-American Public Health, PAHO, WHO
- 2012 Elected Member, The Association of American Physicians (AAP)
- 2012 The Ralph D. Feigin, M.D. Award for Excellence, The Immunization Partnership
- 2013 Vaccine Nation, Top 50 most influential vaccine personalities
- 2013 David Pakcard Lecturer, Uniformed Services University of the Health Sciences
- 2013 Joseph F. Russo MD Lectureship, North American Society for Pediatric and Adolescent Gynecology
- 2013 JV Irons Keynote Lecture, The 63rd Annual James Steele Conference on Diseases in Nature
- 2013 Edward Kass Lectureship, IDWEEK - Infectious Diseases Societies in America Conference

C. Selected peer-reviewed publications (in chronological order). (Selected from 284 in PubMed)

1. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm Infection. *N Engl J Med* 2004; 351:799-807.
2. Asojo OA, Goud G, Dhar K, Loukas A, Zhan B, Deumic V, Liu S, Borgstahl GE, Hotez PJ. X-ray structure of Na-ASP-2, a pathogenesis-related-1 protein from the nematode parasite, *Necator americanus*, and a vaccine antigen for human hookworm infection. *J Mol Biol* 2005; 346:801-14.
3. Hotez PJ. Neglected Infections of Poverty in the United States of America. *PLoS Negl Trop Dis* 2008; 2:e256. PMID: PMC2430531.
4. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Ehrlich Sachs S, Sachs JD, Savioli L. Control of neglected tropical diseases. *N Engl J Med* 2007; 357: 1018-27.
5. Hotez PJ, Brindley P, Bethony J, King CH, Pearce E, Jacobson J. Helminth infections: the great neglected tropical diseases. *J Clin Invest* 2008; 118: 1311-21. PMID: PMC2276811
6. Bethony JM, Simon G, Diemert DJ, Parenti D, Desrosiers A, Schuck S, Fujiwara R, Santiago H, Hotez PJ. Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 hookworm vaccine in unexposed adults. *Vaccine* 2008; 26: 2408-17.
7. Hotez PJ, Fenwick A, Savioli L, Molyneux DH. Rescuing the "bottom billion" through neglected tropical disease control. *Lancet* 2009; 373: 1570-4.
8. Hotez PJ. Peace through vaccine diplomacy. *Science* 2010; 327: 1301.
www.sciencemag.org/content/327/5971/1301.full?sid=5be8053a-cc9c-4381-a6e9-815c955ba849
9. Zhan B, Perally S, Brophy PM, Xue J, Goud G, Liu S, Deumic V, de Oliveira LM, Bethony J, Bottazzi ME, Jiang D, Gillespie P, Xiao SH, Gupta R, Loukas A, Ranjit N, Lustigman S, Oksov Y, Hotez P. Molecular Cloning, Biochemical Characterization and Partial Protective Immunity of the Heme-binding Glutathione S-Transferases from the Human Hookworm *Necator americanus*. *Infect Immun* 2010; 78:1552-63. PMID: PMC2849424
10. Hotez PJ, Bethony JM, Diemert DJ, Pearson M, Loukas A. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat Rev Microbiol* 2010; 8: 814-26.
11. Hotez PJ, Mistry N, Rubinstein J, Sachs JD. Integrating neglected tropical diseases into AIDS, tuberculosis, and malaria control. *N Engl J Med* 2011; 364: 2086-9.
12. Hotez PJ. A handful of 'antipoverty' vaccines exist for neglected diseases, but the world's poorest billion people need more. *Health Aff (Millwood)* 2011; 30: 1080-7. PMID: 21653960.
13. Goud GN, Deumic V, Gupta R, et al. Expression, purification, and molecular analysis of the *Necator americanus* glutathione S-transferase 1 (Na-GST-1): a production process developed for a lead candidate recombinant hookworm vaccine antigen. *Protein Expr Purif* 2012; 83: 145-51.

14. Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, Jones K, Zhan B, Hotez PJ. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccines for severe acute respiratory syndrome. *Exp Rev Vaccines* 2012; 11: 1405. PMID: 23252385
15. Hotez PJ, Diemert D, Bacon KM, Beaumier C, Bethony JM, Bottazzi ME, Brooker S, Couto AR, Freire Mda S, Homma A, Lee BY, Loukas A, Loblack M, Morel CM, Oliveira RC, Russell PK. The Human Hookworm Vaccine. *Vaccine* 2013; 31 Suppl 2:B227-32. PMID: 23598487

D. Research Support

Ongoing Research Support

23386 Hotez (PI) 01/01/2011-12/31/2014

Sponsor: Dutch Government

Title: Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the *Na*-GST-1 and *Na*-APR-1 hookworm antigens in both adults and children.

1016395 Hotez (PI) 08/01/2011 – 09/30/2015

Sponsor: Private Source

Title: Human Hookworm Vaccine Initiative 3

Clinical Development and Evaluation of the *Na*-GST-1 and *Na*-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Hotez (PI) 04/20/2012 – 04/19/2016

Private Source

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

NIH R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

Sponsor: National Institutes of Health

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi (Center Director) 10/01/2012 – 12/31/2017

Sponsor: Department of Health and Human Services / Texas A&M Univ.

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development

Role: Instructor

Hotez (PI) 09/01/2013 – 08/31/2017

Sponsor: Private Source

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

HOOKVAC Hotez (PI) 10/01/2013 – 09/30/2017

Sponsor: European Union via sub from Amsterdam Institute for Global Health and Development (AIGHD)

Title: Developing and Testing a novel, low-cost, effective HOOKworm VACCine to Control Human Hookworm Infection in endemic countries

Major goals of the project are to perform technology transfer of processes for fermentation purification and analytical testing of the human hookworm vaccine.

Hotez (PI) 01/01/2014 – 12/31/2016

Sponsor: University of Malaya
Title: Malaysian Neglected Tropical Disease Initiative
Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

Hotez (PI) 01/01/2014 – 12/31/2017
Sponsor: (b)(4)
Title: West Nile Virus vaccine development
Main goal is to support West Nile Virus vaccine development.

Hotez/Bottazzi (MPI) 01/01/2014 – 12/31/2017
Sponsor: (b)(4)
Title: Hookworm Vaccine Discovery Program
The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Bottazzi/Hotez (MPI) 07/01/2012 – 12/31/2013
Sponsor: Instituto Carlos Slim de la Salud
Title: Slim Initiative for Antipoverty Vaccine Development
The main goal of this project is to build a new generation of urgently needed vaccines for the neglected diseases, and to build capacity for vaccine development in Mexico.

Completed Research Support (selected support after 2010)

32472 Hotez (PI) 08/01/2006 – 12/31/2012
Sponsor: Sabin Vaccine Institute is conduit for The Bill and Melinda Gates Foundation
Title: Human Hookworm Vaccine Initiative 1
Human Hookworm Vaccine Initiative (HHVI): Clinical Development & Evaluation of the Na-ASP-2 Hookworm Vaccine
The goal of this study is to continue product development, including the manufacture of a second pilot lot, and to conduct a global health impact analysis of the human hookworm vaccine with the Sabin Vaccine institute

A/ 90577 Hotez (PI) 01/01/2011 – 12/31/2012
Sponsor: National Institutes of Health
Title: Product development of a membrane tetraspanin vaccine against schistosomiasis
The goal of this project is the development of a high-yield, low-cost process for producing and formulating a recombinant Sm-TSP-2 schistosomiasis vaccine (10-liter scale)

RCA8608 Hotez (PI) 09/30/2009 - 09/30/2011
(b)(4) PATH-MVI
Development of a Malaria transmission blocking Vaccine
To develop a feasibility of expression study for the expression and scale-up of the Anopheles-APN-1 target candidate antigen as a malaria transmission blocking vaccine. The goal of this study is to conduct the early feasibility of expression using both yeast and bacterial expression systems.

38988 Hotez (PI) 08/01/2006 - 07/31/2011
Human Hookworm Vaccine Initiative 2
Sabin Vaccine Institute-Human Hookworm Vaccine Initiative (HHVI): To develop and test the Na-APR-1 Hookworm Vaccine
The goal of this study is to conduct the process development, cGMP manufacture and testing, and clinical evaluation of APR-1 in order to develop a bivalent human hookworm vaccine with the Sabin Vaccine Institute

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Asojo, Oluwatoyin Ajibola	POSITION TITLE Associate Professor		
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
Trent University, Peterborough ON Canada	B.Sc.	05/92	Chemistry & Economics
Trent University, Peterborough ON Canada	B.Sc. Hons	05/93	Chemistry
University of Houston, TX	Ph.D.	12/99	Chemistry
NCI-SAIC Frederick, MD	Post doc	05/00	Crystallography

A. Personal Statement

I am currently Associate Professor at the National School of Tropical Medicine (NSTM) at Baylor College of medicine and collaborate with Dr. Hotez on the SARs grant. I will mentor the high school students placed at laboratories at NSTM. I am an URM female and over the course of my career I have mentored a diverse group of high school, pre-doctoral, and medical students. These students have played an integral part in my research and many of them have been co-authors on papers generated from my laboratory. I have served as a principal investigator or collaborator, on extramural contracts and grants, from the NIH, and DOD. I have unique skills and experiences that allow me to tackle innovative ideas and utilize diverse sources of information as evident from the diversity of projects I have worked on. I was initially trained as a chemist with my research area mainly in structural biology. As a structural biologist I have solved protein structures from bacteria, protozoa, viruses, human, (cancer) and nematodes. I have also carried out rational drug development studies on diverse targets. These experiences allowed me to develop an integrated view of science and see correlations in these complex biological systems that would not have been apparent if I had a more limited research portfolio. I look forward to mentoring the student.

B. Positions and Honors

Positions and Employment

1999-2000	Fellow, Structural Biology Program National Cancer Institute, Frederick, MD
2000-2001	Structural Biologist Tibotec-Virco, Rockville MD
2002-2003	Fellow, Macromolecular Crystallography Program National Cancer Institute Frederick MD
2002-2012	Scientific Collaborator Human Hookworm Vaccine Initiative GWU Washington DC
2003-2005	Research Assistant Prof. Eppler Institute University of Nebraska Medical Center, Omaha NE
2005- 2012	Assistant Prof. Pathology and Microbiology University of Nebraska Medical Center Omaha NE
2012-	Associate Professor, National School of Tropical Medicine, Baylor College of Medicine, Houston

Other Experience and Professional Memberships

1999-	American Chemical Society (Omaha Local section past chair/ chair / chair elect 2008 – 2011)
1999-	American Crystallographic Association (Member)
2005-	American Association for Cancer Research (Member)
2012-	American Society of Tropical medicine and Hygiene (Member)
2005-	Peer-Reviewer ACTA Cryst-D/F,
2005-	Peer Reviewer Advanced Photon Source Macromolecular Crystallography proposals
2005-2007	Edna Ittner Research Grants UNMC
2011-2012	Biophysics Society (Member)
2009	Ad hoc Reviewer ARRA National Science Foundation
2011	Ad hoc Reviewer NIH study section
2013	Reviewer AACR Minority in Cancer Award
2013	NIH Bioinformatics to Mine Human Bio-specimens Data Workshop

2001, 2008- American Chemical Society Project SEED coordinator & mentor
 2013- BCM Medical School Admissions Committee, BCM IMSD Committee,
 2012- ABRCMS, SANCS Reviewer
 2013 BCM Pediatrics Pilot Grants Reviewer
 2013- ACS Chemistry Ambassador, Rice University Civic Scientist
 2014- Ad hoc Reviewer NSF BIO

Honors

2013- CAPES-Fiocruz/CDTS Fellowship Program Visiting Professorship to Brazil
 2013-2015 Master's Teacher's Fellowship Program
 2012 AACR Minority in Cancer Research Award
 2006 Univ. Nebraska Omaha Women of Color Award in Science and Technology
 2005 Keystone Symposia scholarship
 1994 -1999 Welch Pre-doctoral Fellowship University of Houston, Houston TX
 1992 -1993 Dean's Honors List Graduating Class Trent University
 1989 -1993 Trent International Program Full Scholarship Trent University Peterborough ON Canada
 1987 -1989 United World College Scholarship Pearson United World College Vancouver BC Canada

C. Selected Peer-reviewed Publications (high school students are highlighted)

1. S. K. Nelson, A. Kelleher, **G. Robinson**, S. Reiling and O. A. Asojo, Crystal structure of 2-Keto-3-deoxy-D-manno-octulosonate-8-phosphate synthase from *Pseudomonas aeruginosa* *Acta Cryst F* Sept 2013 F.
2. O. A. Asojo*, G. N. Goud, B. Zhan, **K. Ordóñez**, M. Sedlacek, K. Homma, V. Deumic, R. Gupta, J. Brelsford, M. K. Price, M. Ngamelue and P. J. Hotez Crystallization and preliminary X-ray analysis of Na-SAA-2 from the human hookworm parasite *Necator americanus*. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 66, 172-6, 2010.
3. Y. Lee, S. Mootien, C. Shoen, M. Destefano, P. Cirillo, O. A. Asojo, K. R. Yeung, M. Leidizet, P. A. Aristoff, R. A. Koski, P. A. Kaplan, K. Anthony, Inhibition of Mycobacterial Alanine Racemase Activity and Growth by Thiadiazolidinones *Biochem Pharmacol*. 2013 May 13. pii: S0006-2952(13)00288-8. doi: 10.1016/j.bcp.2013.05.004.
4. Bai, O. A. Asojo, P. Cirillo, M. Ciustea, M. Ledizet, P. A. Aristoff, L. Leng, R. A. Koski, T. J. Powell, R. Bucala, K. G. Anthony* A novel allosteric inhibitor of macrophage migration inhibitory factor (MIF). *J. Biol Chem* 2012 Jul 10. [Epub ahead of print]. PMID: PMC3436310
5. O. A. Asojo* Structure of a two-CAP domain protein from the human hookworm parasite *Necator americanus* *Acta D* 2011 Vol 67(Pt 5), pages 455-62. PMID: PMC3087624
6. O. A. Asojo*, R. A. Koski & N. Bonafé Structural studies of human glioma pathogenesis-related protein 1 *Acta D* 2011 Vol 67(Pt 10), p 847-855. PMID: PMC3176621
7. S. A. Reiling, K. Homma, & O. A. Asojo*, Purification and crystallization of RNase HIII from *Staphylococcus aureus* *Acta Cryst.* (2011). **F67**, 79-82. PMID: PMC3079978
8. N. Bonafé, B. Zhan, M. E. Bottazzi, O. A. Perez, R. A. Koski, O. A. Asojo*, Expression, purification, crystallization and preliminary X-ray analysis of a truncated soluble domain of human glioma pathogenesis-related protein 1. *Acta Crystallogr Sect F Struct Biol Cryst Commun*. 2010 Nov 1;66 (Pt 11):1487-9. Epub 2010 Oct 28 2010. PMID: PMC3001655
9. M. Ngamelue, K. Homma, O. Lockridge, O. A. Asojo*, Crystal structure of recombinant full length human butyrylcholinesterase *Acta Cryst.* **F63**, 723–727, 2007. PMID: PMC2376307
10. O. A. Asojo*, K. Homma, **M. Sedlacek**, M. Ngamelue, G. N. Goud, B. Zhan, V. Deumic, O. Asojo, P. J. Hotez. X-ray structures of NaGST-1 and NaGST-2 two glutathione s-transferase from the human hookworm *Necator americanus*. *BMC Structural Biology*, 7:42, 2007. PMID: PMC1924862
11. O. A. Asojo*, E. Schott, G. Vasta and A. M. Silva, X-ray structures of PmSOD1 and PmSOD2, Two Superoxide Dismutases from the Protozoan Parasite *Perkinsus marinus*. *Acta Cryst F*, Nov 1;62(Pt 11):1072-5, 2006. PMID: PMC2225229
12. O. A. Asojo*, G. Goud, K. Dhar, A. Loukas, B. Zhan, V. Deumic, S. Liu, G.E. Borgstahl, P. J. Hotez, X-ray structure of Na-ASP-2, a Pathogenesis Related-1 protein from the nematode parasite *Necator americanus* and a vaccine antigen for human hookworm infection *J. Mol Biol.*, 346(3), 801-814, 2005.
13. Nachon*, O. A. Asojo, G.E. Borgstahl, P. Masson, and O. Lockridge, Role of Water in Aging of Human Butyrylcholinesterase Inhibited by Echothiophate: The Crystal Structure Suggests Two Alternative Mechanisms of Aging. *Biochemistry* 44, 1154-1162, 2005.

14. O. A. Asojo, C. Boulegue, D. M. Hoover, W. Lu, and J. Lubkowski*. High resolution structures of thymus and activation-regulated chemokine (TARC) *Acta Cryst.*, D59, 1165-1173, 2003.
15. O. A. Asojo*, S. Gulnik, E. Afonina, T. S. Haque, J. A. Ellman, and A. M. Silva*. Novel uncomplexed and complexed structures of plasmepsin II, a protease from *Plasmodium falciparum*. *J. Mol Biol.*, 327:173-181, 2003.

D. Research Support

Ongoing Research Support

5U01AI082081-04 Anthony (PI) 05/01/12-04/30/14
NIH/NIAID sub-award from L2 Diagnostics
Broad-Spectrum Antimicrobials Targeting the D-Alanine Pathway
Project objectives: To identify small molecule inhibitors of the cell wall synthesis enzyme, alanine racemase for therapeutic development of a broad spectrum antibacterial against a variety of bacterial pathogens.
Role: Investigator

2R44AR056908-02A1 Anthony (PI) 10/01/12 – 09/30/15
NIH via subaward from L2 Diagnostics
Therapeutic inhibition of MIF in Rheumatoid Arthritis
The goal of this project is to characterize the precise mechanism of novel inhibitors of hMIF that are being developed as therapeutics for Rheumatoid Arthritis.
Role: Sub-PI

Hotez (Center Director, Consultant) 07/01/12 – 12/31/17
Sponsor: Department of Health and Human Services / Texas A&M Univ.
Title: Centers for Innovation in Advanced Development and Manufacturing
The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.
Role: Instructor

Past Research Support (last 3 years)

5K01CA113486 Asojo (PI) 06/03/2005 -05/31/2011
Structural Basis of Multidrug resistance in Cancer (No cost-extension 05/31/2010 -05/31/2011)
NCI Mentored Career Development Award for Underrepresented Minorities: the major goals of this project are to transition into an independent researcher and to characterize the structures and functions of the human multi-drug transporter ABCG2. Role: PI

Department of Defense Bayles (PI) 04/01/2009 – 03/30/2011
Fighting drug resistance infections
My role on this project is to carry out structural studies of CidA and LrgA, programmed cell death regulatory proteins. Role: Co-PI

SUMMARY OF THE PROJECT SPECIFIC AIMS AND APPROACH

Funds are requested for a minority supplement to the parent grant AI-098775 on the development and manufacture of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV). The project will serve as a basis for engaging an under-represented minority high school student in an eight-week long mentored program of molecular biology and biochemistry research. The program will be offered in association with the Office of Diversity and Community Outreach at Baylor College of Medicine.

SPECIFIC AIMS. Briefly, the parent grant seeks to develop a recombinant vaccine against SARS, now classified by NIAID as a Category C Priority Pathogen, by advancing the vaccine through early stage expression and preclinical characterization, process development, formulation, stability, technology transfer and cGMP manufacture. The Actual Specific Aims of the parent grant are as follows:

Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. We will evaluate the expression of the recombinant RBD (rRBD) in bacteria and yeast expression systems and select one of them for expression of rRBD protein for subsequent studies based on yields, purity, stability, antigenicity, functionality, immunogenicity, and efficacy (for inducing neutralizing antibody responses and protection against SARS-CoV challenge) of the rRBD protein when formulated in alum. We will use the rRBD protein from the selected expression system for optimization of immunization regimens, and assess the ability of rRBD protein formulated with alum based adjuvants and/or GLA, a TLR4 antagonist adjuvant, to induce cross-neutralizing antibody response, cross-protection and long-term immune responses and protection in mouse models using the optimized immunization regimen. (Timeline Year 1-3)

Specific Aim 2: Process development, characterization, formulation and stability profiling. In parallel to Aim 1, a scalable and reproducible fermentation process for rRBD (10 liter scale) and a purification process using chromatographic technologies will be developed. Reproducibility will be confirmed and specific product quality assays will be developed and used to characterize the recombinant vaccine protein. Vaccine buffer formulations will be developed and characterized using an innovative approach, combining analytical/biochemical tools with biophysical assays to test different excipients and stabilizers and establish an optimal stability profile. The stabilized protein will be formulated with alum and/or GLA. The binding and effect on the structure stability will be examined. Immunogenicity and efficacy of the vaccine formulations will be evaluated in parallel as described in Aim 1. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. (Timeline Year 2-4)

Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation. The cell bank production, production processes and formulation technology for the selected rRBD-based vaccine will be transferred to Walter Reed Army Institute of Research (WRAIR) pilot facility for 60-L scale GMP manufacture, formulation and fill and finish. The clinical lots will be released by BCM-Sabin and following a pre-IND meeting with the U.S. FDA, GLP toxicology will be initiated at Frontier Biosciences, a Maryland-based contractor. BCM-Sabin will prepare and submit an IND in preparation for the initiation of the clinical development plan. (Timeline Year 4-5)

With the exception of the final cGMP manufacture and preclinical testing for immunogenicity and efficacy using a mouse challenge model all of the work is conducted at the Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine (BCM-Sabin).

APPROACH. The strengths of our approach rely on an extensive evidence base of preliminary data collected over the last seven years by our collaborators on the grant at the New York Blood Center (NYBC), which point to the RBD protein as a lead candidate vaccine antigen, together with an eleven-year track record of product development and testing for recombinant vaccines by the Sabin Vaccine Institute Product Development Partnership now based at Baylor College of Medicine (BCM-Sabin). Additionally, BCM-Sabin has had previous success in tech transferring processes for recombinant protein vaccines to Walter Reed Army Institute of Research, our cGMP contractor (CMO) of choice. Further, the group has demonstrated the feasibility of developing rRBD-based subunit vaccines. Specifically, our collaborators at NYBC have shown that mammalian cell-expressed rRBD fused induces high titer of RBD-specific neutralizing antibodies in vaccinated animals and long-term (over a year) immune responses and protection against subsequent SARS-CoV challenge, while using the pseudotyped viruses expressing S protein originated from Tor2, GD03, or SZ3, the representative strains of human 2002–2003 & 2003–2004 SARS-CoV and palm civet SARS-CoV, respectively, we also found that mouse and rabbit antibodies raised against rRBD derived from either one of the aforementioned strains of SARS-CoV and the palm civet SARS-CoV cross-neutralize one another, suggesting their protective efficacy against challenge with heterogeneous viruses. Immunization of mice with rRBD derived from various expressing systems, including mammalian cells (293T and CHO), insect sf9 cells, and *E. coli* is capable of producing high-levels of RBD-specific neutralizing antibody and potent T cell responses against both pseudotyped and live SARS-CoV. Importantly, these rRBD proteins appear to maintain intact conformation and authentic antigenicity, reacting with the RBD-specific and conformation-dependent mAbs with neutralizing activity. These rRBD proteins elicited immunity that protected all vaccinated mice from SARS-CoV challenge. These preliminary data and expertise by NYBC is now being paired with the track record at BCM-Sabin/WRAIR for transitioning a recombinant protein-based vaccine through process development and formulation, technology transfer for cGMP manufacture, lot release and stability testing, and GLP toxicology testing. All steps needed in order to compile a regulatory file application (IND) in preparation for a submission to the FDA. .

The Major Deliverables
1. Development of a scalable, reproducible process for expression of the rRBD protein and formulation of the RBD-based vaccine with high quality and stability. 2. Establishment of an immunization regimen, including the optimized antigen dose, vaccination schedule and route and adjuvant formulation, for evaluation of the immunogenicity and efficacy of the vaccine. 3. Successful pilot cGMP manufacture of a subunit SARS vaccine comprised of rRBD protein and an adjuvant formulation. 4. Completion of the GLP toxicology testing with acceptable safety profile. 5. IND preparation and submission with U.S. FDA.

The parent grant was written and submitted in response to an RFA in biodefense - RFA-AI-11-014 Partnerships for Biodefense (R01) - BCM-Sabin is applying its proven product development partnership approach and strong peer-reviewed publication track record in research and development of parasitic recombinant protein vaccines to accelerate the development of a recombinant RBD-based vaccine to prevent SARS-CoV infection. To ensure success, BCM-Sabin provides program management throughout the project timeline and leverage the collaborations with the research and industry partners to augment the expertise for anti-SARS-CoV vaccine testing. The strengths of this proposal relay on the supporting proof of concept data that identifies the RBD protein as a lead candidate vaccine antigen selected for further process development, characterization and preclinical evaluation. Following feasibility of expression in both bacterial and yeast expression systems, the recombinant proteins are assessed at BCM-Sabin for scalability, yield, quality and stability. Antigenicity, functionality, immunogenicity and potency of the vaccine formulations will be developed and evaluated at NYBC and additional collaborators at University of Texas Medical Branch Galveston (UTMB). Furthermore, rRBD protein from the selected expression system will be compared at NYBC and UTMB for its ability to induce neutralizing antibodies and protection in laboratory animals against challenge infections with multiple strains of the SARS-CoV using different adjuvant platforms; alum (either Alhydrogel® or aluminum phosphate) and/or GLA, a TLR4 antagonist adjuvant. Once the most efficacious expression system is selected, BCM-Sabin will perform scale-up process development (PD) at the 10 liter fermentation scale followed by protein purification under detailed documentation. Reproducibility will be confirmed and specific product quality control assays will be developed in collaboration with NYBC and used to characterize rRBD. In

addition, vaccine buffer formulations will be developed and characterized, and excipients and stabilizers will be evaluated. The stabilized molecule will then be formulated with alum and/or GLA and their effect on the structure stability will also be examined. These assays and procedures will serve the basis for formal lot release and stability evaluation post-manufacturing. The developed process will then be transferred to WRAIR that successfully manufactured two hookworm vaccines with BCM-Sabin. After pilot lot material is produced and released and following a pre-IND meeting with the U.S. FDA, toxicology testing will commence and an IND application will be prepared. Thus a key strength is clear-cut project deliverables (see adjoining box).

The proposed project is ideal for mentoring a under-represented highschool student on fundamental techniques in molecular biology and biochemistry and providing a broad educational overview on the key steps in developing a vaccine to prevent a major public health threat.

PLAN/TIMELINE FOR RESEARCH AND CAREER DEVELOPMENT (INCLUDING MENTORSHIP ACTIVITIES)

We have planned for an 8 week period of research. During this period of research, the highschool student will meet with our research team regularly to observe research meetings, and also contribute to the meetings once they start collecting data. The student will be mentored through the entire research period and trained in various general aspects of research. Each week the student will have a one-on-one mentorship meeting where they can give feedback on their activities for the week and point out any areas that they may not understand, or may need additional training with.

The detailed RESEARCH AND MENTORSHIP PLAN is described below with a set of targets for each week:

Week 1:

- Laboratory orientation and rules
- Overview of Ethical guidelines in research – explanation of human subjects and animal protections
- Introduction to molecular biology and biochemistry, with emphasis on recombinant DNA technologies
- Overview of Professionalism
- Training in basic knowledge of nucleic acids, proteins, and respiratory viruses
- Attendance of laboratory meeting (group)
- Review of the research strategy and timelines (with Principal Investigator)

Weeks 2-6

Training and orientation on basic techniques in molecular biology and biochemistry

- How to prepare buffers and reagents
- How to prepare and run agarose gels and visualize and quantitate DNA
- How to use restriction endonucleases
- How to prepare plasmids and transfect yeast and bacteria
- How to induce protein expression and analyze recombinant proteins on SDS-PAGE

Week 2:

- Record manipulations and observations – Preparation of buffers and reagents; agarose gels and visualizing and quantitating DNA
- Attendance of laboratory meeting (group)
- Overview of the National Institutes of Health
- Mentorship meeting (one-on-one)

Week 3:

- Record manipulations and observations – use of restriction endonucleases and ligases
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training on how to use empirical data in preparing a research project
- Training in how to find empirical data and how to organize it

Week 4:

- Record manipulations and observations –preparing plasmids and yeast and bacterial transfection
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training in scientific writing and on how to write an abstract

Week 5:

- Record manipulations and observations –preparing plasmids and yeast and bacterial transfections
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training in the history and function of the IRB

Week 6:

- Record manipulations and observations – protein expression and analysis of recombinant proteins on SDS PAGE
- Attendance of laboratory meeting (group)
- Mentorship meeting (one-on-one)
- Training in oral presentation skills

Week 7:

- Record manipulations and observations – protein expression and analysis of recombinant protein on SDS PAGE.
- Evaluation of experiments and data collection
- Introduction to creating a research poster
- Attendance of laboratory meeting (group)
- Produce a draft presentation of research findings with the assistance of mentor (one-on-one)

Week 8:

- Meetings with PI and laboratory staff to review results
- Finalize draft of research findings
- Presentation of findings at laboratory meeting
- Provide feedback on how to improve laboratory experiences
- Reflect on the summer research experience

Evidence of mentoring experience for the Principal Investigator:

Peter Hotez MD PhD - the Principal Investigator - is an elected member of the Institute of Medicine with more than 25 years of experience leading laboratory investigations on neglected and infectious diseases. Over the last decade he has led a non-profit product development partnership for the development, manufacture, and clinical testing of recombinant protein vaccines to combat neglected tropical diseases, and was recently named one of the fifty most influential people in vaccines. Dr. Hotez has supervised or co-supervised at least a dozen postdoctoral fellows and a dozen research faculty, in addition to three PhD candidates for their doctoral dissertations (including an MD PhD student now in training). He has also supervised the thesis work of numerous students for their master degree in public health (MPH) and related degrees. He is widely sought out by medical and graduate students, as well as undergraduates for career advice.

For this mentored program much of the direct training at the laboratory bench will be led by senior laboratory staff members and research-intensive faculty working in the larger laboratory group of Dr. Hotez. Similar arrangements have led to numerous successful mentoring experiences for other high school students and undergraduates. Last summer, two high school students received laboratory instruction and training in the Hotez laboratory.

Description of how the research and career development experiences will expand and foster the research capabilities of the candidate:

The described course of summer study will introduce the student to the basic principals of biomedical scientific research. The student will understand the planning process and the approaches that are used to develop a project, analyze the data, and in the preparation of data for presentation. The candidate/student will gain a basic understanding of molecular biology and biochemistry of macromolecules (nucleic acids and proteins) and how these approaches contribute to our understanding of the mechanisms that allow respiratory viruses to invade host tissues and how vaccines may be used to combat them. The student will gain experience in laboratory techniques, procedures, biosafety and ethics.

Under the guidance of experienced researcher the student will learn fundamental techniques in molecular biology and biochemistry including buffer and reagent preparation, agarose gels and DNA visualization and quantitation, use and application of restriction endonucleases, preparation of plasmids and transfection of yeast and bacteria, fusion protein induction and expression, and analysis of recombinant proteins on SDS-PAGE. These studies will be conducted in the larger context and environment of the steps needed to develop and test recombinant protein vaccines for important infectious agents and biodefense threats including SARS. Thus we hope to stimulate the imagination of the trainee about the role he/she could one day have in the important area of public health emergency preparedness.

Equally important, this student will observe the scientific process unfold in a laboratory setting. We anticipate that the student will become engaged in the practice and discipline of academic research and, since this is biomedical research, come to understand and appreciate the role of research in the practice of medicine. The overall goal is for this high school student is to see the challenges and opportunities that exist in arenas of higher education. One of the goals is to instill a sense of awe and excitement in the student as to all of the possibilities available to them in their future. It is our objective to provide this student with world-class training and experience in an 8 week defined course of study.

In conjunction with the Office of Diversity and Community Outreach at Baylor College of Medicine, the student will present a research poster at the annual student research symposium that this office sponsors each year in January.

Statement of Eligibility from the Investigator

Ebe Ewere [redacted] is a sophomore at Hightower High School in Missouri City, Texas, and is a U.S. citizen [redacted]. We hope that the early exposure of this candidate to this area of scientific research and the experience he will gain by participating in a laboratory study will further influence his decision to pursue a career in the healthcare field, therefore impacting the national scientific workforce. This experience will also be beneficial to Ebe as he begins to make decisions about the pursuit of his undergraduate studies. Additionally, this experience will provide a better understanding of the relationship between research studies and the delivery of healthcare, as well as basic research methods. His presence here at Baylor in the capacity of project intern for the summer will further enhance our institution's goal of diversity, and its goal to have an impact in reducing the racial and ethnic disparity in the scientific and healthcare workforce. Ebe Ewere has not received any previous or current PHS support.



Peter Hotez, MD, PhD
Principal Investigator
Dean of School of Tropical Medicine
Baylor College of Medicine



James L. Phillips, MD
Senior Associate Dean
Office of Diversity and Community Outreach
Baylor College of Medicine



Leanne B. Scott, Ph.D.
Director, Sponsored Programs

BIOGRAPHICAL SKETCHFollow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Ewere, Ebe Aaron		POSITION TITLE	
eRA COMMONS USER NAME (last four digits of social) P11			
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
Hightower High School's Medical Science Academy	N/A	06/16	N/A

NOTE: The Biographical Sketch may not exceed four pages. Follow the formats and instructions below.

A. Personal Statement

I first recognized my calling into a scientific career as a fifth grader when I was introduced into the wonderful sport of American football. I developed a newfound admiration for the human body and the way that it works. I realized that the teamwork between the muscles, bones and nerves within a successful athlete's body paralleled the teamwork between teammates on a successful football team. While in seventh grade, I had the opportunity to play on a team which further exposed me to the beauty of teamwork. Teamwork's importance and impact was everything I could have imagined and more, further engrossing me in the science behind the teamwork on the field as well as the teamwork occurring within my body. After one memorable season, I refrained from playing competitive football; however, my admiration for the human body continues to grow unceasingly.

My interest in the human body grew to include learning about the medical sciences. Anatomy, physiology, and psychology are some of my favorite subjects and I enjoy reading about them just for the sake of becoming more knowledgeable. However, as I got older, I found that I was not entirely satisfied with just being knowledgeable. The information that I obtained is relevant, but I wanted to be able to apply it. I heard about Fort Bend ISD's Medical Science Academy and knew that it was my golden opportunity to further my knowledge of medicine, apply it, and obtain a centralized academic focus in an area that I am so passionate for. I applied to the academy, received acceptance, and graciously took my first major step towards fulfilling my dreams of having a career in medicine.

Since enrolling in the Medical Science Academy, each day spent in my medical class has been a constant reconfirmation of where my passion lies. Being engrossed in the medical sciences has shown me many aspects of what the medical field really consists of and with that, I have grown to see that there are certain aspects that I like much more than others. Ever since I was on the football team in seventh grade, I told myself and others that I wanted to be an orthopaedic surgeon, but learning about the many different fields under medicine has led me to find many other topics of medicine, such as neurology, to be as interesting as orthopedics.

My interest in neurology has led me on a path of progressive research on a disease called cerebral palsy. The types of cerebral palsy that are most intriguing to me are hypertonic and ataxic cerebral palsy. My research has gotten me in contact with Dr. Iona Novak, a professor at University of Notre Dame in Australia who is also the head of the Cerebral Palsy Alliance. We discuss via email, and she has been a great asset to my research and understanding of the disease due to her structured guidance and various resources that she shares with me. Currently, there is no cure for cerebral palsy, but there are numerous methods of treatments and preventive measures that mothers can take to lower the risk of having a child with cerebral palsy such as

the administration of intravenous magnesium sulfate. Despite the current status of there being no known cure for cerebral palsy, I feel that with adequate research and experimentation, a cure could be discovered. If I choose to become a neurologist in the future, my greatest goal would be to discover the cure to such a debilitating disease. As much as I am attracted to neurology and orthopedics, I am still open to learning about other disciplines of medicine because I am aware that I need further exposure to truly know which area I am most passionate about.

Being a project intern in Baylor College of Medicine's Summer Research Program would give me access to various fields of medical science which will give me the needed exposure to help me discover in which field I should pursue a career. I also would hope to gain an understanding of what professional research is, engage in the application of my previously acquired medical knowledge through hands on training, and also expand and develop my knowledge base. I feel that participation in Baylor College of Medicine's Summer Research Program is a once in a lifetime experience. I truly believe that to be granted such an opportunity will benefit me in numerous ways and that it will place me in a position to help others as I apply my medical knowledge in making scientific discoveries.

B. Positions and Honors

Positions

2009 - 2010	Member of Quail Valley Middle School's Science Club
2009 - 2012	Student of Fort Bend ISD's Academy for the Gifted and Talented at Quail Valley
2010 - Present	Member of Grace International Church's Media and Technical Team
2010 - 2011	Member of the Quail Valley Middle School Football Team
2011 - 2012	Member of the Quail Valley Middle School Basketball Team
	Member of the i9 Sports Winter Basketball League Champion team
2012 - Present	Student of Hightower High School's Medical Science Academy
2012 - 2013	Class President of Medical Terminology Class at Hightower High School
2012 - Present	Chapter Historian for Health Occupations Students of America (HOSA)
	Recruiter for Grace International's Camera and Technical Team
Spring 2013	Freshman representative for Hightower High School at Fort Bend ISD's Annual Diversity Conference
2013 - Present	Class President of Anatomy and Physiology Class at Hightower High School
	Member of the Science UIL Club
	Registered Volunteer with City Wide Club
	Member of Bini Club of Houston, Inc.'s Youth Drama Team
	Member of Grace International Church's "Heavenly Steppers" Dance Team
2014 - Present	Sophomore representative for Hightower High School at Fort Bend ISD's Annual Diversity Conference
	Member of Baylor College of Medicine's Saturday Morning Science Program

Honors

2009 - 2010	Participant in the Mars Rover Competition
2010 - 2011	Participant in the Science Fair Competition - Chemistry Project
2011 - 2012	League leader in points per game and steals per game in i9 sports Winter Basketball League
	Participant in the Science Fair Competition - Physics Project
2012 - 2013	Recipient of the Academic Excellence Award
2012 - 2013	Recipient of the Most Improved Student of the Year Award - awarded by Pre-AP Spanish III Instructor

Program Director/Principal Investigator (Last, First, Middle):

2013 - 2014 Participant in the Science Fair Competition – Physics Project
Nominated for the Outstanding Sophomore Award
Participant in the Science Fair Competition – Physics Project

Signature:

Elbe Gwerc

C. Selected Peer-reviewed Publications

N/A

D. Research Support

N/A

Page 222 of 352

Withheld pursuant to exemption

Personal Info

of the Freedom of Information and Privacy Act



To whom it may concern:

Ebe Ewere (Name of Student), a student from Hightower High School
(Insert name of school), located at 3333 Hurricane lane, missouri city TX 77459 (school address), would like to participate in the summer research experience offered to them through the Saturday Morning Science program at Baylor College of Medicine for the summer of 2014. I understand that said student will be working in a research lab under the direction of a Principal Investigator at Baylor College of Medicine who is operating under an active grant funded through the National Institutes of Health.

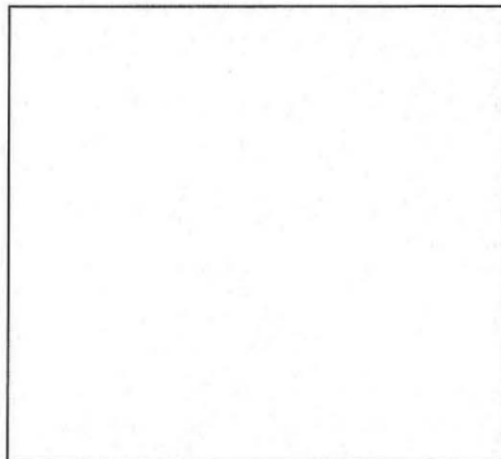
I approve of Ebe Ewere's (name of student) participation in this research program, and I attest that their participation will not detract from or interfere with the student's course of studies.

Name of school official completing document: Lester Johnson

Role of school official: Associate Principal Phone #: 281 634 5279

Signature of school official: [Signature] Date: 2/27/14

Please affix school stamp or seal in the designated space below (if no stamp or seal please have school official sign with school name and address):



Ebe Ewere

Personal Info

Education

Hightower High School's Medical Science Academy

Cumulative GPA:

Anticipated graduation date: June 2016

Experience

Research-Based

- 2009 Participant in the Mars Rover Competition
- 2010 - 2011 Participant in the Science Fair Competition – Chemistry Project
- 2011 – 2012 Participant in the Science Fair Competition – Physics Project
- 2012 – 2013 Participant in the Science Fair Competition – Physics Project
- 2014 – Present Member of Baylor College of Medicine's Saturday Morning Science Program

Leadership-Based

- 2012 - Present Chapter Historian for Health Occupations Students of America
- 2012-2013 Class President of Medical Terminology Class at Hightower High School
- 2013 Freshman representative for Hightower High School at Fort Bend ISD's Annual Diversity Conference
- 2013 - Present Recruiter for Grace International's Media and Technical Team
- 2013 - Present Class President of Anatomy and Physiology Class at Hightower High School
- 2014 - Present Sophomore representative for Hightower High School at Fort Bend ISD's Annual Diversity Conference

Activities

- 2009 - 2010 Member of Quail Valley Middle School's Science Club
- 2010 - 2011 Member of i9 Sports Winter Basketball League
- 2010 - Present Member of Grace International Church's Media and Technical Team
- 2013 – 2014 Member of the Science UIL Club
- 2010 - 2011 Member of the Quail Valley Middle School Basketball Team

2013 – Present Registered Volunteer with City Wide Club

2013 – Present Member of Bini Club of Houston, INC.'s youth drama team

2013 – Present Member of Grace International Church's "Heavenly Steppers" Dance Team

Honors

2011-2012 League leader in points per game and steals per game in i9 sports Winter Basketball League

2012-2013 Recipient of the Academic Excellence Award

2012-2013 Recipient of the Most Improved Student of the Year Award (from Spanish 3 Pre-AP class)

2013-2014 Nominated for the Outstanding Sophomore Award

Skills

Five years of Spanish education

Skilled with the Android and Microsoft operating systems

Adept with Microsoft Office Software

Experienced with still, digital and video cameras

Proficient in touch typing



NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 4R01AI098775-05
FAIN: R01AI098775

Principal Investigator(s):
Maria Elena Bottazzi, PHD
PETER J HOTEZ (contact), PHD
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Leanne Brooks Scott
Business Official
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Period Of Performance:
Budget Period: 05/01/2016 – 04/30/2017
Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$1,165,855 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Ann W. Devine
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 4R01AI098775-05**Award Calculation (U.S. Dollars)**

Federal Direct Costs	\$768,645
Federal F&A Costs	\$397,210
Approved Budget	\$1,165,855
Total Amount of Federal Funds Obligated (Federal Share)	\$1,165,855
TOTAL FEDERAL AWARD AMOUNT	\$1,165,855

AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$1,165,855
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SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
5	\$1,165,855	\$1,165,855

Fiscal Information:

CFDA Name:	Allergy, Immunology and Transplantation Research
CFDA Number:	93.855
EIN:	1741613878A1
Document Number:	RAI098775B
PMS Account Type:	P (Subaccount)
Fiscal Year:	2016

IC	CAN	2016
AI	8472315	\$1,165,855

NIH Administrative Data:**PCC:** M51C B / **OC:** 414E / **Released:** Pii 04/07/2016**Award Processed:** 04/09/2016 12:00:53 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 4R01AI098775-05

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 4R01AI098775-05

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Award.
- b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- c. 45 CFR Part 75.
- d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
- f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm> for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal

Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This award was issued as a non-competing continuation with a change in document number. This change was made solely to accommodate the HHS mandate to transition award payments to Payment Management System (PMS) subaccounts. Expenses for the project period should be treated as if this were a non-competing continuation award (e.g. Type 5). A Subaccount Transitional Federal Financial Report (FFR) is required for the previous budget periods to complete the transition. Recipients must use the SF-425 as they would for an interim or annual FFR and enter "Subaccount Transitional FFR" in box 12. This report covers grant funds in the pooled account and is used by NIH to end the grant's association with the pooled PMS payment account and transition award payments to the PMS subaccount established for the grant, including transferring any carryover funds and unliquidated obligations remaining in the pooled account. It is due within 90 days of the end of the calendar quarter in which the last budget period prior to this NoA ended. For more information, please refer to Notice NOT-OD-15-105

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI098775. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: <http://grants.nih.gov/grants/policy/policy.htm#gps>.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, <http://grants.nih.gov/grants/policy/policy.htm#gps>, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not

required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: <http://grants.nih.gov/grants/forms.htm>. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, R13, R25, S10.

Unless an application for competitive renewal is submitted, a final progress report must also be submitted within 120 days of the expiration date. Instructions for preparing a Final Progress Report are at: <http://grants.nih.gov/grants/funding/finalprogressreport.pdf>. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH strongly encourages electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final progress report and final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final progress report and the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final Progress Report is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than \$10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

SECTION IV – AI Special Terms and Conditions – 4R01AI098775-05

This award includes funds awarded for consortium activity with NY Blood Center.
This award includes funds awarded for consortium activity with the University of Texas Medical Branch.

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps/HTML5/section_15/15_consortium_agreements.htm.

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Adam Graham

Email: adam.graham@nih.gov **Phone:** 301-761-5430 **Fax:** 301-493-0597

Program Official: Erik J. Stemmy

Email: erik.stemmy@nih.gov **Phone:** 240-627-3380

SPREADSHEET SUMMARY

GRANT NUMBER: 4R01AI098775-05

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	Year 5
TOTAL FEDERAL DC	\$768,645
TOTAL FEDERAL F&A	\$397,210
TOTAL COST	\$1,165,855

Facilities and Administrative Costs	Year 5
F&A Cost Rate 1	57.3%
F&A Cost Base 1	\$693,211
F&A Costs 1	\$397,210

A. COVER PAGE

Project Title: RBD recombinant protein-based SARS vaccine for biodefense	
Grant Number: 5R01AI098775-05	Project/Grant Period: 05/04/2012 - 04/30/2017
Reporting Period: 05/01/2015 - 04/30/2016	Requested Budget Period: 05/01/2016 - 04/30/2017
Report Term Frequency: Annual	Date Submitted: 03/15/2016
Program Director/Principal Investigator Information: PETER J HOTEZ , MD PHD BA Phone number: 832-824-0502 Email: hotez@bcm.edu	Recipient Organization: BAYLOR COLLEGE OF MEDICINE BAYLOR COLLEGE OF MEDICINE 1 BAYLOR PLAZA HOUSTON, TX 770303411 DUNS: 051113330 EIN: 1741613878A1 RECIPIENT ID: 35116-N4
Change of Contact PD/PI: N/A	
Administrative Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu	Signing Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu
Human Subjects: Yes HS Exempt: Yes Exemption Number: E4 Phase III Clinical Trial:	Vertebrate Animals: Yes
hESC: No	Inventions/Patents: Yes If yes, previously reported: Yes

B. ACCOMPLISHMENTS**B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?**

The major goals of the project are: Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate (Timeline Year 1-3). Specific Aim 2: Process development, characterization, formulation and stability profiling (Timeline Year 2-4) and Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation (Timeline Year 4-5).

As proposed, for this reporting period activities related to Specific Aim 1 (Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate) were initiated. Specifically, we have achieved 50% completion of the activities related to the sub-specific aims 1.A. Feasibility of scalable expression, 1.B. Antigenicity and functionality and 1.C. Immunogenicity. For sub-specific aim 1.D. Efficacy, 33.3% of this activity has been completed. The goals will not change for the next reporting period and no significant changes in approach or methods are envisioned.

B.1.a Have the major goals changed since the initial competing award or previous report?

Yes

Revised goals:

The major goals for the project are: Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate (Timeline Year 1-3). Specific Aim 2: Process development, characterization, formulation and stability profiling (Timeline Year 2-4) and Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation (Timeline Year 4-5).

Previously completed sub-specific aims: 1.A. (Feasibility of scalable expression), 1.B. (Antigenicity and functionality), 1.C. (Immunogenicity), 1.D. (Efficacy), 1.E. (Optimization of the immunization regimens), 2.A. (Development and optimization of a 10 L scale process (fermentation & purification)) and 2B. (Assay development).

For this reporting period, activities related to Specific Aim 1 (Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate) were advanced. Specifically, we have achieved 85% completion of activities related to the sub-aims 1.F. (Efficacy of rRBD-based vaccine to induce cross-protection in mice) and 1.G. (Efficacy of the rRBD-based vaccine to induce long-term immune responses and protection).

Activities related to Specific Aim 2 (Process development, characterization, formulation and stability profiling) were also advanced. Specifically, we have achieved 100% completion of sub- aim 2.C. (Execution of three successive process development runs at the 10 L scale). For sub- aim 2.D. (Formulation and stabilization), 85% of that activity has been completed.

As originally proposed for this reporting period, activities related to Specific Aim 3 (Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation) were initiated. Specifically, we have achieved 40% completion of the activities related to the sub-aim 3.A. (Strategy for Manufacture of Drug Substance and Drug Product). For sub-aims 3.B. (Lot release and start of a stability program) and 3.C. (IND Regulatory package preparation and submission), 30% of these activities have been completed.

A request of a Change of Scope was submitted to NIAID on January 15, 2016 and is awaiting approval to modify the specific aims to be completed during Year 5 of this application. The Consortium proposes to complete the 60-L scale GMP manufacture and testing currently ongoing for the SARS RBD vaccine. However, instead of performing the SARS RBD vaccine GLP toxicology and IND preparations, these activities would be replaced with MERS vaccine development activities including the expression, purification and feasibility-of-scale-up evaluation of the MERS-CoV rRBD protein vaccine and its preclinical characterization. In addition, we would evaluate the MERS vaccine antigenicity, immunogenicity and efficacy.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

The Co-I was invited to present an update on the status of current work to the weekly meeting of the Division of Microbiology and Infectious Disease at the National Institutes of Health National Institute of Allergy and Infectious Diseases (NIH/NIAD) on December 17, 2015. The title of the presentation was "Vaccine Development against Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome."

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

As we move into year five, we plan to confirm the optimal adjuvant formulation of the selected yeast-expressed, rRBD protein-based SARS vaccine candidate RBD219-N1 combined with Alhydrogel®. Formulations of the RBD219-N1 protein combined in various ratios of Alhydrogel® will be compared. Alhydrogel® alone will be used as the control. We will assess augmentation of RBD-specific immunogenicity (IgG, IgG1, IgG2a antibody responses) and elicitation of effective neutralizing antibodies in the vaccinated mice using ELISA, as well as pseudo-type and live SARS-CoV-based neutralization assays. In addition, we will test the survival of immunized mice after virus challenge and for the possibility of the development of pathology in the lungs of vaccinated mice.

We will continue using product quality assays and formal stability evaluations to conduct lot release and post-manufacturing stability testing. Likewise, we will maintain our scheduled quality assurance activities with our associates at the Sabin Vaccine Institute as we prepare regulatory documents.

We will continue to closely coordinate with the consortium partners NYBC and UTMB.

The NYBC partners plan to further develop specific assays for evaluating the potency of the SARS-CoV RBD protein, RBD219-N1. The potency of the RBD protein will be evaluated by its ability to induce high titers of neutralizing antibodies in sera of mice immunized i.m. twice with 3 different doses (10, 5, 2.5 µg) of alum-formulated RBD protein at a fixed ratio of 1:8. In addition, the antigenic and functional potencies of the RBD protein, using the same amount used for mouse immunization, will also be determined quantitatively by its ability to bind to SARS-CoV RBD-specific mAb 33G4, and to SARS-CoV's receptor angiotensin converting enzyme 2 (ACE2) using ELISA platform assays. The correlation among the three RBD's potencies; the ability to induce neutralizing antibodies; and the ability to bind mAb 33G4 as well as the receptor ACE2, will be analyzed. If these parameters are significantly correlated, this particular lot of the RBD219-N1 protein will be used as the standard vaccine antigen, and the ELISA assays for measuring the binding of RBD protein to the mAb 33G4 will become the standard assay for evaluating the potency of newly produced RBD219-N1 proteins.

The UTMB partners will complete the preclinical evaluation of the optimal adjuvant formulation of the selected yeast-expressed, rRBD protein-based SARS vaccine candidate RBD219-N1 combined with Alhydrogel® as previously described.

A request of a Change of Scope was submitted to NIAID on January 15, 2016 and is awaiting approval to modify the specific aims to be completed during Year 5 of this application. The Consortium proposes to complete the 60-L scale GMP manufacture and testing currently ongoing for the SARS RBD vaccine. However, instead of performing the SARS RBD vaccine GLP toxicology and IND preparations, these activities would be replaced with MERS vaccine development activities including the expression, purification and feasibility-of-scale-up evaluation of the MERS-CoV rRBD protein vaccine and its preclinical characterization. In addition, we would evaluate vaccine antigenicity, immunogenicity and efficacy.

B.2 Year 4 Accomplishments

As described in last year's report, the immunization route for the selected SARS vaccine candidate RBD219-N1 was optimized (Aim 1) as were the 10 L scale process and assay development (Aim 2). For this reporting period, further progress was made toward completing Aim 1. Other major activities performed were in support of completing Aim 2 and advancing Aim 3.

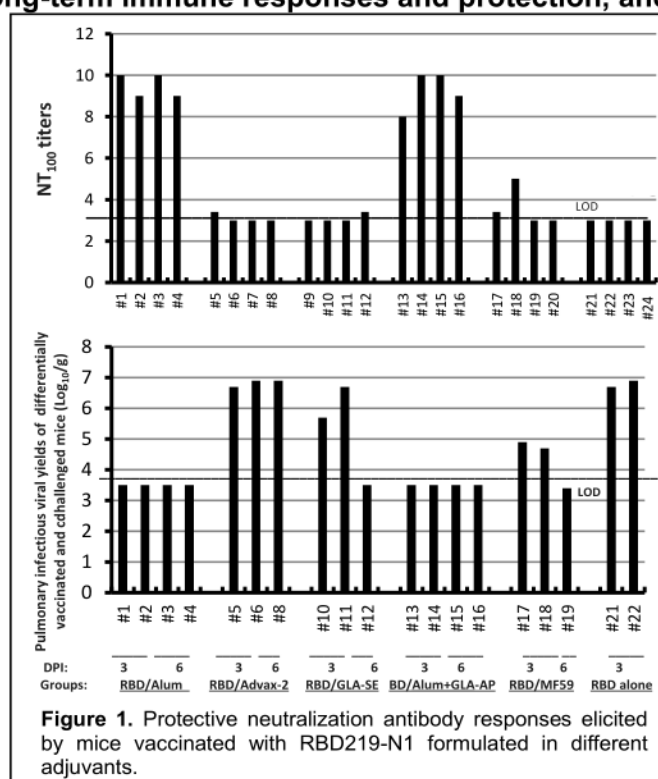
Aim 1.F. Ability of the rRBD-based vaccine to induce cross-protection in mice.

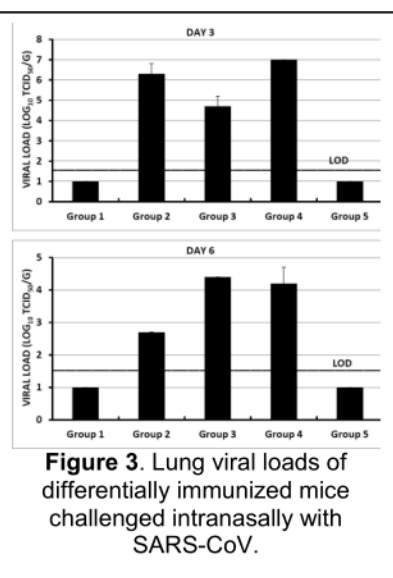
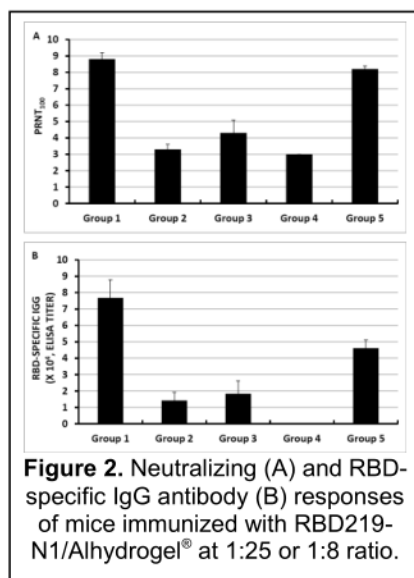
The cross-neutralizing activity and long-term immunogenicity of the SARS-CoV RBD protein, RBD219-N1, were evaluated by testing the sera of mice immunized with alum-formulated RBD219-N1. We have shown that the RBD219-N1 vaccine induced highly potent anti-RBD neutralizing antibodies that also cross-neutralized divergent strains of SARS pseudovirus expressing SARS-CoV spike (S) proteins isolated at the early (Tor2 strain) and late stage (GD03 strain) of the 2003-4 SARS pandemic, as well as civet SARS-CoV (SZ3 strain). The RBD219-N1 vaccine further was able to induce long-term high levels of neutralizing antibodies against the SARS pseudovirus; high titers were maintained over a 7-months period. These data confirm the excellent potency of the SARS-CoV RBD vaccine against SARS-CoV infection.

Aim 1.G. Ability of the rRBD-based vaccine to induce long-term immune responses and protection, and Aim 2.D. Formulation and Stabilization.

To select the optimal adjuvant for the formulation of the RBD219-N1 vaccine, we compared several adjuvants (Alhydrogel[®] [aluminum hydroxide], Advax-2 [Inulin plus CpG], glucopyranosyl lipid adjuvant-stable emulsion [GLA-SE], glucopyranosyl lipid adjuvant-aqueous formulation [GLA-AF] plus Alhydrogel[®], and MF59) with regard to their ability to improve the efficacy of RBD219-N1 vaccine in mice. Briefly, we immunized 6 groups of *Balb/c* mice (4 per group, 3 times, 3-weeks apart) via the intramuscular (i.m.) route. Mice were bled 10 days after the last immunization for testing the specific antibody responses before intranasal (i.n.) challenge with SARS-CoV. We sacrificed two mice in each group at days 3 and 6 post infection (p.i.) for determining the lung pathology and viral loads. As shown in Figure 1, mice immunized with RBD219-N1 or SARS-CoV S protein formulated with Alhydrogel[®] produced potent neutralizing antibody responses, resulting in complete protection against subsequent SARS-CoV infection. In contrast, we noted formulations with Advax-1, GLA-SE, MF59, or Tris buffer alone failed to elicit protective neutralizing antibody responses, resulting in incomplete protection against SARS-CoV infection, as evaluated by the isolation of infectious virus and quantitative PCR (qPCR) for viral RNA. We also noted that RBD219-N1 formulated with Alhydrogel[®] was the only vaccine formulation which did not cause eosinophilic infiltration within the lungs (data not shown). Taken together, these results suggest that RBD219-N1/Alhydrogel[®] is an effective and safe vaccine for SARS-CoV infection and disease.

Although RBD219-N1 formulated on Alhydrogel[®] at a ratio of 1:25 has been proven effective in immunized animals against lethal SARS-CoV challenge without causing eosinophilic-type pulmonary immunopathology, such a high RBD219-N1-Alhydrogel[®] ratio is prohibitive in evaluating large dose ranges given the FDA's allowance of aluminum in vaccines. Therefore, we compared the efficacy of RBD219-N1 formulated on Alhydrogel[®] at either 1:25 or 1:8 ratios in mice using the same scheme described above. Five groups (N=6) were studied: Group 1) RBD219-N1/Alhydrogel[®] (RBD: 20, 10, 10 μ g; Alhydrogel[®]: 500, 250, 250 μ g, a ratio of 1:25), Group 2) RBD219-N1/Alhydrogel[®] (RBD: 20, 10, 10 μ g; Alhydrogel[®]: 160, 80, 80 μ g, a ratio of 1:8), Group 3) RBD219-N1/Alhydrogel[®] (RBD: 10, 10, 10 μ g; Alhydrogel[®]: 80, 80, 80 μ g, a ratio of 1:8), Group 4) Tris-Alhydrogel[®] as control (Alhydrogel[®]: 160, 160, 160 μ g), and Group 5) SARS-CoV/Alhydrogel[®] (S: 3, 3, 3 μ g; Alhydrogel[®]: pre-formulated). The sera were collected 10 days after the last vaccination to test RBD219-N1-specific IgG antibody responses by ELISA and neutralizing antibodies against live SARS-CoV infections. Results showed that mice immunized with RBD219-N1/Alhydrogel[®] at 1:25 produced significantly





higher neutralizing antibody and RBD219-N1-specific IgG titers than those formulated at the 1:8 ratio (Figure 2A-B). Consistent with the antibody responses, mice immunized with RBD219-N1/Alhydrogel[®] (1:25) were completely protected against lethal challenge with SARS-CoV, as indicated by undetectable infectious virus within the lungs and also a lack of morbidity and mortality (Figure 3). In contrast, infectious virus was recovered from the lungs of mice immunized with a 1:8 ratio of RBD219-N1/ Alhydrogel[®] (Groups 2 and 3) by day 5 p.i and one mouse from each group died. As expected, mice immunized with Alhydrogel[®] alone (Group 4) were not protected against lethal viral challenge and had detectable virus in the lungs resulting in the death of 2 mice on

day 4 p.i. While SARS-CoV S protein formulated on Alhydrogel[®] (Group 5) was also immunogenic and protective against SARS-CoV infection, histopathology examination of lung tissues consistently revealed enhanced infiltration of eosinophils (data not shown).

The difference in findings of the groups that received the 1:8 ratio compared to the 1:25 ratio was unexpected as at a 1:8 ratio of RBD219-N1/ Alhydrogel[®] the protein is completely adsorbed to the Alhydrogel[®] and it is not predicted that an excess of unbound recombinant protein, such as in the 1:25 ratio groups, should have a favorable impact on the vaccine efficacy. At this point, we consider these data to be inconclusive and are planning to repeat and expand the preclinical formulation studies in Year 5.

Aim 2.C. Execution of three successive process development runs at the 10 L scale.

We continued the optimization of our upstream and downstream processes, and proceeded with the three successive process development runs at 10 L scale. This approach using three independent process runs was used to ensure robustness of the process and yield unbiased results. The summary for the three runs (Table 1) shows the process was reproducible with a variance of less than 15%.

Run #	Volume of FS (L)	Rough yield of FS (mg RBD/L of FS)	Final Concentration (mg RBD/mL)	Final yield (mg RBD/L of FS)	Process Recovery (%)
Run 1	5.8	424	1.80	250	54
Run 2	5.5	427	1.38	201	48
Run 3	5.9	424	1.68	228	54
Mean	5.7±0.2	425±1.6	1.62±0.22	226±25	52±3
%CV	3.5%	0.4%	13.4%	10.9%	7.0%

FS – indicates Fermentation Supernatant

Characterization of the purified RBD219-N1 from these three runs was performed using the developed assays (Aim 2.B.), including SDS-PAGE, Western Blot, Silver Staining, HPLC-PR, pH, A280 and Color and

Appearance. Based on the characterization, the three lots of RBD219-N1 were very similar with purity ≥95%. An accelerated stability study was conducted to monitor RBD219-N1 stability at different temperatures (4°C, room temperature and 37°C) over a month. The stability study results suggested the RBD219-N1 from three successive runs remained stable even at 37°C for a month.

Aim 3.A. Strategy for Manufacture of Drug Substance and Drug Product

Upon completion of the three reproducible process development runs, we arranged the technology transfer for cGMP Manufacture by providing a research clone for the generation of Master and Production Cell Banks. With the assistance of our quality assurance associates at the Sabin Vaccine Institute, batch production records were reviewed and finalized to ensure equitable procedures would be utilized by the authorized contract manufacturing organization for the final fermentation and purification of the RBD219-N1.

Aim 3.C. IND Regulatory Package Preparation and Submission

Preliminary work has begun on the preparation of a pre-IND briefing package by compiling information from the pre-clinical work for the CMC.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?**B.4 What opportunities for training and professional development has the project provided?**

Under the guidance of an experienced researcher, an under-represented minority high school student spent eight weeks learning and practicing the fundamental techniques in molecular biology and biochemistry while assisting with process development of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV).

The student assisted with the development of downstream (purification) process thereby contributing to the optimization of a reproducible 10 L scale process for a stable rRBD-based vaccine. The student was also able to assist in supporting the three reproducible product development runs in preparation for future technology transfer to a cGMP manufacturing facility.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Du L, Jiang S. Middle East respiratory syndrome: current status and future prospects for vaccine development. Expert Opin Biol Ther. 2015;15(11):1647-51. PubMed PMID: 26414077; PubMed Central PMCID: PMC4636333.
Complete	Honda-Okubo Y, Barnard D, Ong CH, Peng BH, Tseng CT, Petrovsky N. Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology. J Virol. 2015 Mar;89(6):2995-3007. PubMed PMID: 25520500; PubMed Central PMCID: PMC4337527.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	HOTEZ, PETER J	PII		BA,PHD, MD	PD/PI	EFFORT	0	0			NA
	Y	Bottazzi, Maria Elena			BS,PHD	PD/PI		0	0			NA
	Y	JIANG, SHIBO			MD,PHD	PD/PI		0	0	Fudan University	CHINA	NA
	N	Tyagi Kundu, Rakhi			MS	Technician		0	0			NA
	N	Agrawal, Anurodh			PhD	Technician		0	0			NA
	N	Poongavanam selvamani, Mohan vivekanandan			PhD	Technician		0	0			NA
	N	Chag, Shivali			MS	Non-Student Research Assistant		0	0			NA
	Y	Tseng, Chien-Te K			PHD,MS	Co-Investigator		0	0			NA
	N	Chan, Tehseng			MD, PhD	Co-Investigator		0	0			NA
	Y	Du, Lanying			PHD	Co-Investigator		0	0			NA
	Y	LUSTIGMAN, SARA			PHD	Co-Investigator		0	0			NA
	N	Nino, Diane			BSci	Project Manager		0	0			NA
	N	Peng, Bi-Hung			PhD	Faculty		0	0			NA
	N	Han, Song			PhD	Research Associate III.		0	0			NA
	N	Tao, Xinrong			Ph.D.	Research Associate		0	0			NA

Glossary of acronyms:

S/K - Senior/Key
 DOB - Date of Birth
 Cal - Person Months (Calendar)
 Aca - Person Months (Academic)
 Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation

SS - Supplement Support

RE - Reentry Supplement

DI - Diversity Supplement

OT - Other

NA - Not Applicable

D.2 PERSONNEL UPDATES**D.2.a Level of Effort**

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

Yes

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D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

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D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Zhan, Bin

eRA COMMONS USER NAME (agency login): eRA Commons User

Name

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Fujian Medical College, Fuzhou, Fujian	MD	07/1983	Medicine
Chinese Academy of Preventive Medicine, Shanghai, Shanghai	MS	07/1989	Parasitology
University of Texas Medical Branch at Galveston, Galveston, Texas	Postdoctoral Fellow	12/1996	Molecular biology
Yale University, New Haven, CT	Fellow	04/1997	Molecular Parasitology
Yale University, New Haven, CT	Fellow	07/2000	Vaccine development

A. Personal Statement

Soon after I received my MD degree I decided to dedicate myself to fight against parasitic diseases and other infectious diseases after I realized millions people in my homeland China and other developing countries were suffering from these diseases. Since then, I have been involved in multidisciplinary studies of epidemiology, immunology, and molecular biology of malaria, leishmania, hookworm and other Neglected Tropical Diseases, especially with interest in the molecular basis of host-parasite interactions and targeting those crucial molecules for the parasitism as vaccine candidates. Particularly, I have focused my research on developing human hookworm vaccines since 2000 when the Human Hookworm Vaccine Initiative (HHVI) was funded by

Private Source

My research includes the molecular cloning of novel hookworm antigens by immunological and molecular biological approaches, characterizing their biological functions in the survival of hookworm in the host as well as evaluating their potential as protective antigens and/or vaccines. My work also involves the animal models for parasites and their life cycle maintenance and pre-clinical vaccine trials. From my work, more than 40 new hookworm antigens as well as their DNA have been identified and cloned from different species and stages of hookworms. Many of them have been characterized and tested for their vaccine potential. Some of these vaccine candidates are in process development and pilot manufacture for clinic trials. In addition to the vaccine development against hookworm, I also work for developing vaccines for other parasitic infections such as malaria, leishmaniasis, Ascariasis, Trichuriasis, Chagas disease, Onchocerciasis and SARS. In addition, For the past four years, I have involved in the design, cloning and expression of receptor-binding protein (RBD) of SARS coronavirus as a vaccine against SARS. I am intensively involved in developing recombinant protein expression in E. coli, yeast and baculovirus/insect cells and have a lot of experiences with these expression platforms. These researches have led to more than 90 publications in peer-reviewed journals.

Given my intensive experiences in protein expression and vaccine development against parasitic infections and SARS, I am confident that I can further contribute myself into the vaccine development against SARS/MERS using my expertise.

B. Positions and Honors

Positions and Employment

1983 - 1986	Assistant lecture, Fujian Medical College, Fuzhou
1989 - 1994	Assistant Professor, Chinese Academy of Preventive Medicine, Institute of Parasitic Diseases, Shanghai
1997 - 2000	Associate Professor, Chief of Pharmacology Department, Chinese Academy of Preventive Medicine, Institute of Parasitic Diseases, Shanghai
2000 - 2003	Assistant Research Professor, The George Washington University , Washington, DC
2004 - 2008	Associate Research Professor, The George Washington University , Washington, DC
2009 - 2010	Research Professor, The George Washington University , Washington , DC
2011 -	Associate Professor, Baylor College of Medicine, Houston, TX

C. Contribution to Science

1. **Hookworm antigen discovery and characterization:** In order to understand the strategies that hookworm utilizes to survive in the intestine of host and the interaction between hookworm and host, A great arsenal of hookworm secreted proteins or surface antigens were identified and cloned by immunoreaction with protective immune sera or other molecular biological approaches. The biological functions of these hookworm antigens were characterized. A total of more than 40 new hookworm antigens as well as their DNA has been identified and cloned from different species and stages of hookworms. Many of them have been characterized and tested for their vaccine potential.
 - a. **Zhan B**, Hotez PJ, Wang Y, Hawdon JM. A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of proteases. *Mol Biochem Parasitol.* 2002 Apr 9;120(2):291-6.
 - b. **Zhan B**, Liu Y, Badamchian M, Williamson A, Feng J, Loukas A, Hawdon JM, Hotez PJ. Molecular characterisation of the *Ancylostoma*-secreted protein family from the adult stage of *Ancylostoma caninum*. *Int J Parasitol.* 2003 Aug;33(9):897-907..
 - c. **Zhan B**, Wang Y, Liu Y, Williamson A, Loukas A, Hawdon JM, Xue HC, Xiao SH, Hotez PJ. Ac-SAA-1, an immunodominant 16 kDa surface-associated antigen of infective larvae and adults of *Ancylostoma caninum*. *Int J Parasitol.* 2004 Aug;34(9):1037-45.
 - d. Jiang D, **Zhan B**, Mayor RS, Gillespie P, Keegan B, Bottazzi ME, Hotez P. Ac-AP-12, a novel factor Xa anticoagulant peptide from the esophageal glands of adult *Ancylostoma caninum*. *Mol Biochem Parasitol.* 2011 May;177(1):42-8.
2. **Vaccine development against hookworm and other parasite infections:** In order to evaluate the vaccine efficacy for those antigens I identified and cloned from hookworm, these antigens were expressed as recombinant proteins and immunized in dog or hamster animal models. The worm reduction was measured against challenge with hookworm infective larvae. Many of them showed effective protection against challenge and some of them have been chosen for clinical trials such as Na-ASP-2, Na-GST-1, Na-APR-1).
 - a. Mendez S, **Zhan B**, Goud G, Ghosh K, Dobardzic A, Wu W, Liu S, Deumic V, Dobardzic R, Liu Y, Bethony J, Hotez PJ. Effect of combining the larval antigens *Ancylostoma* secreted protein 2 (ASP-2) and metalloprotease 1 (MTP-1) in protecting hamsters against hookworm infection and disease caused by *Ancylostoma ceylanicum*. *Vaccine.* 2005 May 2;23(24):3123-30..
 - b. **Zhan B**, Liu S, Perally S, Xue J, Fujiwara R, Brophy P, Xiao S, Liu Y, Feng J, Williamson A, Wang Y, Bueno LL, Mendez S, Goud G, Bethony JM, Hawdon JM, Loukas A, Jones K, Hotez PJ. Biochemical characterization and vaccine potential of a heme-binding glutathione transferase from the adult hookworm *Ancylostoma caninum*. *Infect Immun.* 2005 Oct;73(10):6903-11.
 - c. **Zhan B**, Perally S, Brophy PM, Xue J, Goud G, Liu S, Deumic V, de Oliveira LM, Bethony J, Bottazzi ME, Jiang D, Gillespie P, Xiao SH, Gupta R, Loukas A, Ranjit N, Lustigman S, Oksov Y, Hotez P. Molecular cloning, biochemical characterization, and partial protective immunity of the heme-binding glutathione S-transferases from the human hookworm *Necator americanus*. *Infect Immun.* 2010 Apr;78(4):1552-63..
 - d. Hess JA, **Zhan B**, Bonne-Année S, Deckman JM, Bottazzi ME, Hotez PJ, Klei TR, Lustigman S, Abraham D. Vaccines to combat river blindness: expression, selection and formulation of vaccines against infection with *Onchocerca volvulus* in a mouse model. *Int J Parasitol.* 2014 Aug;44(9):637-46.

3. **Vaccine development against SARS/MERS:** Except for hookworm vaccine, I also devote myself into vaccine development against SARS and MERS. I am intensively involved in developing recombinant protein expression in *E. coli*, yeast and baculovirus/insect cells expression platforms to produce recombinant proteins for preclinical vaccine trials and immunodiagnosis.
 - a. Chen WH, Du L, Chag SM, Ma C, Tricoche N, Tao X, Seid CA, Hudspeth EM, Lustigman S, Tseng CT, Bottazzi ME, Hotez PJ, Zhan B, Jiang S. Yeast-expressed recombinant protein of the receptor-binding domain in SARS-CoV spike protein with deglycosylated forms as a SARS vaccine candidate. *Hum Vaccin Immunother*.
 - b. Hotez PJ, Bottazzi ME, Tseng CT, Zhan B, Lustigman S, Du L, Jiang S. Calling for rapid development of a safe and effective MERS vaccine. *Microbes Infect*. 2014 Jul;16(7):529-31. PubMed PMID: [24931059](#).
 - c. Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, Jones K, Zhan B, Hotez PJ. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. *Expert Rev Vaccines*. 2012 Dec;11(12):1405-13. PubMed PMID: [23252385](#); PubMed Central PMCID: [PMC3586247](#)
 - d. **Zhan B**, Ajmera R, Geiger SM, Gonçalves MT, Liu Z, Wei J, Wilkins PP, Fujiwara R, Gazzinelli Guimaraes PH, Bottazzi ME, Hotez P. Identification of immunodominant antigens for the laboratory diagnosis of toxocariasis. *Trop Med Int Health*. 2015 Dec;20(12):1787-1796.
4. **Hookworm animal models for vaccine and drug trials:** Many efforts have devoted to maintain hookworms in dog (*Ancylostoma caninum*) or in hamsters (*Necator americanus* and *A. ceylanicum*) as models for testing vaccine and drug trials. These models have led to several successful vaccine and drug products.
 - a. Jian X, Shu-Hua X, Hui-Qing Q, Sen L, Hotez P, Bing-Gui S, Hai-Chou X, Tie-Hua L, **Bin Z**. *Necator americanus*: maintenance through one hundred generations in golden hamsters (*Mesocricetus auratus*). II. Morphological development of the adult and its comparison with humans. *Exp Parasitol*. 2003 Nov-Dec;105(3-4):192-200.
 - b. Xiao S, **Zhan B**, Xue J, Goud GN, Loukas A, Liu Y, Williamson A, Liu S, Deumic V, Hotez P. The evaluation of recombinant hookworm antigens as vaccines in hamsters (*Mesocricetus auratus*) challenged with human hookworm, *Necator americanus*. *Exp Parasitol*. 2008 Jan;118(1):32-40.
 - c. Xue J, **Zhan B**, Guo J, He N, Qiang HQ, Hotez P, Xiao SH. Acquired hookworm immunity in the golden hamster (*Mesocricetus auratus*) elicited by living *Necator americanus* third-stage infective larvae. *Exp Parasitol*. 2012 Jan;130(1):6-12.
 - d. Hu Y, **Zhan B**, Keegan B, Yiu YY, Miller MM, Jones K, Aroian RV. Mechanistic and single-dose in vivo therapeutic studies of Cry5B anthelmintic action against hookworms. *PLoS Negl Trop Dis*. 2012;6(11):e1900.
5. **Immunomodulation of nematode-secreted proteins:** Parasitic nematodes secrete a lot of proteins with roles in immunomodulating host immune response as a survival strategy, simultaneously, infected hosts get benefits from these proteins in balancing their immune system. Some of these nematode-secreted proteins were identified and used for treatment of allergic or autoimmune diseases.
 - a. **Zhan B**, Badamchian M, Meihua B, Ashcom J, Feng J, Hawdon J, Shuhua X, Hotez PJ. Molecular cloning and purification of Ac-TMP, a developmentally regulated putative tissue inhibitor of metalloprotease released in relative abundance by adult *Ancylostoma* hookworms. *Am J Trop Med Hyg*. 2002 Mar;66(3):238-44.
 - b. **Zhan B**, Gupta R, Wong SP, Bier S, Jiang D, Goud G, Hotez P. Molecular cloning and characterization of Ac-TMP-2, a tissue inhibitor of metalloproteinase secreted by adult *Ancylostoma caninum*. *Mol Biochem Parasitol*. 2008 Dec;162(2):142-8.
 - c. **Zhan B**, Santiago H, Keegan B, Gillespie P, Xue J, Bethony J, de Oliveira LM, Jiang D, Diemert D, Xiao SH, Jones K, Feng X, Hotez PJ, Bottazzi ME. Fusion of Na-ASP-2 with human immunoglobulin Fc γ abrogates histamine release from basophils sensitized with anti-Na-ASP-2 IgE. *Parasite Immunol*. 2012 Aug-Sep;34(8-9):404-11.
 - d. Yang X, Yang Y, Wang Y, **Zhan B**, Gu Y, Cheng Y, Zhu X. Excretory/secretory products from *Trichinella spiralis* adult worms ameliorate DSS-induced colitis in mice. *PLoS One*. 2014;9(5):e96454.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/bin.zhan.1/bibliography/43438454/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

2015/01/01-2017/12/31

0000, [Private Source] Peter Hotez (PI)

Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease
The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.
Role: KP

2012/07/31-2017/12/31

0000, Department of Health and Human Services / Texas A&M Univ; Peter Hotez (PI)

Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.
Role: OP

2013/01/15-2017/12/31

1R01AI105431-01, NIH via New York Blood Center; Sara Lustigman (PI)

Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.
Role: Co-Investigator

2014/01/01-2017/12/31

0000, [Private Source] Hotez/Bottazzi (PI)

Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.
Role: Co-Investigator

2013/09/01-2017/08/31

0000, [Private Source] Peter Hotez (PI)

Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate Ascaris antigen and a Trichuris antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.
Role: Co-Investigator

02/03/2015-01/31/2020

1 R01 CA183984-01A1, NIH/NCI; Qizhi Cathy Yao

A Novel miR-198 replacement therapy for pancreatic cancer

The main goal of this project is Mesothelin-targeted nanoparticle delivery of gemcitabine for pancreatic cancer
Role: Co-Investigator

Completed Research Support

2009/08/25-2015/07/31

1 R01A1078314 03 , NIH/NIAID (Sub-award from New York Blood Center); Sara Lustigman (PI)

The development of a recombinant vaccine against human onchocerciasis

The major goal of this subcontract is clone, express, characterize and optimize the expression of the eight selected *Onchocerca* vaccine candidate antigens (rOvAgs) using the yeast *Pichia* eukaryotic system.

Role: CPI

2014/08/01-2015/07/31

0000, [Private Source] Zhan/Cheolho (PI)

Anti-filarial vaccine discovery through transcriptome analysis of *Brugia pahangi*

The overarching goal of this one-year project is to identify filarial transmission-blocking vaccine candidates by deep sequencing analysis of the transcriptome of *Brugia pahangi* L3 in mosquito.

Role: CPI

2011/08/01-2015/04/30

1016395, [Private Source] Peter Hotez (PI)

Human Hookworm Vaccine Initiative 3

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Role: KP

2010/08/01-2014/04/30

2R01AI056189 - 07 , NIH; Ravi Aroian (PI)

B. thuringiensis Crystal Proteins as Powerful Next-Generation Anthelmintics

The major goal of this subcontract is to test the effects of different formulated Cry5B against hookworm using *Ancylostoma ceylanicum*/hamster model

Role: CPI

2011/01/01-2013/12/31

0000, Dutch Government ; Peter Hotez (PI)

Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the Na-GST-1 and Na-APR-1 hookworm antigens in both adults and children.

Role: KP

2012/01/01-2012/09/15

HHSN272201001148P, NIH; Michael Bukrinsky (PI)

Immunogenicity and *Leishmania* Vaccine potential of Sand Fly Saliva in Humans

The findings obtained from this proposal will lead to the next steps in early process development of vaccine candidates and demonstrate that proteins can be produced by fermentation and successfully purified and formulated in a consistent and reproducible manner. The ultimate goal is that these basic processes and procedures will be used in downstream cGMP manufacture, GLP toxicology studies and ultimately in the preparation of an Investigational New Drug (IND) application to be submitted to the US Food and Drug Administration in order to commence First-in Human trials.

Role: KP

**OTHER SUPPORT
AT BAYLOR COLLEGE OF MEDICINE**

HOTEZ, PETER J.

New grants awarded:

Instituto Carlos Slim de la Salud 11/1/15 – 10/31/18

Private Source

1/1/15 – 12/31/17

Grant ended:

Dutch Government grant

CURRENT

Hotez (PI) 04/20/2012 – 12/31/2016

Private Source

\$229,534

EFFORT

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

5R01AI098775-02 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

Sponsor: National Institutes of Health \$782,695

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

EFFORT

Bottazzi (Center Director, Consultant) 11/01/2012 – 08/31/2016

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$145,090

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

Role: Instructor

EFFORT

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017

Sponsor: Private Source

\$275,435

EFFORT

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

Hotez (PI) 01/01/2014 – 12/31/2016

Sponsor: University of Malaya \$250,000

Title: Malaysian Neglected Tropical Disease Initiative

Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

EFFORT

Hotez (PI) 01/01/2014 – 12/31/2017

Sponsor: Private Source

\$160,000

EFFORT

Title: West Nile Virus vaccine development

Main goal is to support West Nile Virus vaccine development.

Hotez/Bottazzi (MPI) 01/01/2014 – 07/31/2017

Sponsor: Private Source

\$104,620

EFFORT

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Bottazzi (PI) 07/01/2012 – 10/31/2018

EFFORT

Sponsor: Instituto Carlos Slim de la Salud \$887,044

Title: Slim Initiative for Antipoverty Vaccine Development (Chagas)

The main goal of this project is to bring a Chagas vaccine candidate, Tc24, to a stage of development where pre-IND packages are submitted to the FDA and COFEPRIS. Also an overarching PRINCIPLE is to build human and infrastructure capacity for vaccine development, manufacture, regulatory science, and first in humans clinical testing in Mexico. The major DELIVERABLE is a developed process for refolding TSA-1.

Hotez (PI) 11/1/2014 – 10/31/2016

EFFORT

Sponsor: Private Source

Title: Adjuvant Technologies to Advance Chagas Disease Vaccine Development \$323,651

The overall goal of this project is to develop a new vaccine formulation for Chagas disease consisting of a promising protein-based antigen (Tc24) formulated with a novel TLR4 agonist adjuvant, E6020, which is designed to skew the immune response toward a T_H1 bias and the generation of cytotoxic T cells.

Hotez (PI) 01/01/2015 – 12/31/2017

EFFORT

Sponsor: Private Source

\$582,355

Title: Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease

Main Goal: The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.

OTHER SUPPORT - BOTTAZZI, MARIA ELENA**New grants awarded:**

Instituto Carlos Slim de la Salud 11/1/15 – 10/31/18

Private Source 11/1/15 – 12/31/17

ACTIVE

Hotez (PI)

04/20/2012 – 12/31/2016

EFFORT

Private Source

\$229,319

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

Role: Director of Product Development

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

EFFORT

Sponsor: National Institutes of Health

\$782,695

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi (Center Director, Consultant) 11/01/2012 – 08/31/2016

EFFORT

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$145,090

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

1R01AI105431-01 Lustigman (PI) 01/15/2013 – 12/31/2017

EFFORT

Sponsor: NIH via New York Blood Center

\$137,860

Title: Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Role: Sub-PI

Hotez/Bottazzi (MPI)

08/01/2013 – 07/31/2017

EFFORT

Sponsor: Private Source

\$275,435

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

HOOKVAC Bottazzi (PI)

10/1/2013 – 9/30/2017

EFFORT

Sponsor: European Union via sub from (AIGHD) \$13,377

Title: Developing and Testing a novel, low-cost, effective HOOKworm VACcine to Control Human Hookworm Infection in endemic countries

Major goals of the project are to perform technology transfer of processes for fermentation purification and analytical testing of the human hookworm vaccine.

Hotez (PI)

01/01/2014 – 12/31/2016

EFFORT

Sponsor: University of Malaya

\$250,000

Title: Malaysian Neglected Tropical Disease Initiative

Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

Role: Co-I

RPPR

Hotez (PI) 01/01/2014 – 12/31/2017
 Sponsor: Private Source \$160,000
 Title: West Nile Virus vaccine development
 Main goal is to support West Nile Virus vaccine development.
 Role: Co-I

EFFORT

Hotez/Bottazzi (MPI) 01/01/2014 – 07/31/2017
 Sponsor: Private Source \$104,620

EFFORT

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Bottazzi (Sub-PI) 09/1/2014 – 08/31/2016

EFFORT

Sponsor: Private Source

\$438,396

Title: Adjuvant Technologies to Advance Chagas Disease Vaccine Development

The overall goal of this project is to develop a new vaccine formulation for Chagas disease consisting of a promising protein-based antigen (Tc24) formulated with a novel TLR4 agonist adjuvant, E6020, which is designed to skew the immune response toward a T_H1 bias and the generation of cytotoxic T cells.

Bottazzi (PI) 07/01/2012 – 10/31/2018

EFFORT

Sponsor: Instituto Carlos Slim de la Salud \$887,044

Title: Slim Initiative for Antipoverty Vaccine Development (Chagas)

The main goal of this project is to bring a Chagas vaccine candidate, Tc24, to a stage of development where pre-IND packages are submitted to the FDA and COFEPRIS. Also an overarching PRINCIPLE is to build human and infrastructure capacity for vaccine development, manufacture, regulatory science, and first in humans clinical testing in Mexico. The major DELIVERABLE is a developed process for refolding TSA-1.

Hotez (PI) 01/01/2015 – 12/31/2017

EFFORT

Sponsor: Private Source

\$582,355

Title: Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease

Main Goal: The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.

OTHER SUPPORT – ZHAN, BIN

Private Source

1/1/15 – 12/31/17

ACTIVE

Bottazzi (Center Director, Consultant) 11/01/2012 – 08/31/2016

EFFORT

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$145,090

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

Role: PT Instructor

1R01AI105431-01 Lustigman (PI) 01/15/2013 – 12/31/2017

EFFORT

Sponsor: NIH via New York Blood Center \$137,860

Title: Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Role: Co-Investigator

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

EFFORT

Sponsor: National Institutes of Health \$782,695

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Role: Co-Investigator

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017

EFFORT

Sponsor: Private Source \$275,435

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

Role: Co-Investigator

Hotez/Bottazzi (MPI) 01/01/2014 – 07/31/2017

EFFORT

Sponsor: Private Source \$104,620

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Role: Co-Investigator

Hotez (PI) 01/01/2015 – 12/31/2017

EFFORT

Sponsor: Private Source \$582,355

Title: Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease

Main Goal: The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.

Role: Director, Molecular Biology

Yao (PI) 02/03/2015-01/31/2020

EFFORT

Sponsor: NIH/NCI \$299,000

Title: A Novel miR-198 replacement therapy for pancreatic cancer

The main goal of this project is Mesothelin-targeted nanoparticle delivery of gemcitabine for pancreatic cancer.

Role: Co-Investigator

OTHER SUPPORT AT NEW YORK BLOOD CENTER

Changes for Dr. Lustigman:

NIH (b)(4) were submitted
 OPP1083910; OPP1099849; 1R01AI078314-01A2; OPP1086618; 1R56 1AI101372-01A1: ended
 No changes in all other four previously funded projects

SARA LUSTIGMAN

ACTIVE

1. 1R01AI105431-01 (PI: S. Lustigman) 1/2013 – 12/2017
 NIH/NIAID \$ \$817,780 annual direct (including two subcontracts) (b)(6)

Development of a novel adjuvant for vaccine sparring

Adjuvants are integrated into vaccines to insure their effectiveness and to support antigen sparing. Currently, alum is the only adjuvant licensed in the U.S., but it has had limited effectiveness when used with commercial flu vaccines. Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

2. OPP1017584 (PI: J Sakanari; Co-PI: S. Lustigman) 11/2012 – 10/2017
 Bill & Melinda Gates Foundation \$ 216,428 annual direct (subcontract) (b)(6)

Developing a macrofilaricidal drug for onchocerciasis using Anacor's novel oxaborole technology

A collaborative research effort between the University of California San Francisco Sandler Center, Anacor Pharmaceuticals and LFKRI of the NYBC to discover new drug therapies for the treatment of river blindness (onchocerciasis). The collaboration's goal is to identify a novel, potent macrofilaricidal drug candidate that is capable of killing adult worms.

3. OPP1083910 (PI: T. Nutman; Co-PI: Lustigman) 8/2013 – 3/2016
 Bill & Melinda Gates Foundation \$76,834 annual direct (Subcontract) (b)(6)

Rapid identification of individuals with viable adult female worms of Onchocerca volvulus: a means to the end

To identify host- and parasite-specific biomarker(s) present in human subjects with viable adult females of *Onchocerca volvulus* and to develop and configure rapid point of care methods to detect (or sense) these biomarkers; a necessary step in the progress towards elimination of onchocerciasis.

PENDING

(b)(4)

Pending Support

OVERLAP

None; effort commitments will be adjusted as necessary if pending proposals are awarded.

Changes for Drs. Shibo Jiang and Lanying Du:

SHIBO JIANG

ACTIVE

5R01 AI098775-04 Hotez, Bottazzi, Jiang (PIs)

05/01/2012 – 04/30/2017

EFFORT

NIH/NIAID

RBD recombinant protein-based SARS vaccine for biodefense

Role: MPI

Overlap: None

ACTIVE

5R21 AI111152-02 (Du)

08/05/2014 – 07/31/2016

EFFORT

NIH/NIAID

B. subtilis spore-delivered M2e-FP-based mucosal universal influenza vaccines

Role: Co-Investigator

Overlap: None

PENDING

Pending Support

LANYING DU

ACTIVE

5R21 AI111152-02 (Du)

08/05/2014 – 07/31/2016

EFFORT

NIH/NIAID

B. subtilis spore-delivered M2e-FP-based mucosal universal influenza vaccines

Role: PI

Overlap: None

PENDING

Pending Support

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS**F.3.a Human Subjects**

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

File uploaded: utmb select agent letter.pdf



March 14, 2016

Dear Colleague:

**Environmental Health & Safety
Biological & Chemical Safety Program**
Materials Management Building, 2.112
301 University Blvd.
Galveston, Texas 77555-1111
O 409.772.1781 F 409.772.8921

The University of Texas Medical Branch at Galveston (UTMB) is a select agent registered entity with the U.S. Health and Human Services, Centers for Disease Control and Prevention Division of Select Agents and Toxins (CDC/DSAT) and U. S. Department of Agriculture, Animal and Plant Health Inspection Service, (USDA/APHIS) National Select Agent Program. The University has been inspected by the CDC/DSAT and USDA/APHIS National Select Agent Program for use of HHS Select Agents, Overlap Select Agents and USDA Select Agents.

Per the requirements of 42 CFR 73 the original certificate of registration was issued October 19, 2004, renewal was granted on April 9, 2007, April 1, 2010 and on March 21, 2012 for use of select agents at BioSafety Levels 2, 3, and 4 and Animal BioSafety Levels 3 and 4. Inspection by CDC/DSAT and USDA/APHIS occurred January 12th to 16th 2015, for the current renewal cycle and approval was granted on March 18, 2015 for three years. The Keiller BSL2, BSL3 East and BSL3 West labs and the ACL3, Robert E. Shope BSL4/ABSL4 and irradiator, the Galveston National Laboratory BSL2 and BSL3, ABSL3, BSL3 Enhanced and BSL4/ABSL4, ABSL4 Aerobiology and irradiator facilities are all select agent registered.

The University has been registered with Health and Human Services, Centers for Disease Control and Prevention as a select agent facility since 1997.

A copy of the registration form is attached. The registration number has been redacted for security purposes.

Please feel free to contact me should you require additional information.

Sincerely,

A handwritten signature in black ink that reads "Domenica Zimmerman".

Domenica Zimmerman
BioSafety Officer
Alternate Responsible Official
UTMB Select Agent Program

Certificate of Registration

Entity Name: University of Texas Medical Branch
Address: 301 University Boulevard
Galveston, TX 77555

Registration #:
Effective Date: March 18, 2015
Expiration Date: March 18, 2018

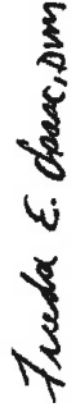
Responsible Official: Michael Shriner

Alternate Responsible Official(s): Carlos Escobar, Danny Jacobs, Scott Weaver, Domenica Zimmerman

Based on information provided to the CDC Select Agent Program and the APHIS Agriculture Select Agent Services, the above named entity is authorized to possess, use, and transfer select agents and toxins under the conditions specified in the entity registration application, in accordance with 42 CFR Part 73, 9 CFR Part 121, and 7 CFR Part 331.



Robbin S. Weyant, Director
Division of Select Agents and Toxins
Centers for Disease Control and Prevention



Freeda E. Isaac, DVM, National Director
Agriculture Select Agent Services
Animal and Plant Health Inspection Service



G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

File(s) uploaded:
utmb select agent.pdf

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: BAYLOR COLLEGE OF MEDICINE	051113330	TX-009	BAYLOR COLLEGE OF MEDICINE ONE BAYLOR PLAZA HOUSTON TX 770303411
New York Blood Center	073271827	NY-014	310 East 67 Street New York NY 100656275
The University of Texas Medical Branch	800771149	TX-014	301 University Boulevard Galveston TX 775550156
Texas Childrens Hospital	074615394	TX-009	1102 Bates Street Houston TX 770302399

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No



Environmental Health & Safety
Biological & Chemical Safety Program
Materials Management Building, 2.112
301 University Blvd.
Galveston, Texas 77555-1111
O 409.772.1781 F 409.772.8921

February 11, 2013

To Whom It May Concern

The University of Texas Medical Branch at Galveston (UTMB) is a select agent registered entity with the U.S. Health and Human Services, Centers for Disease Control and Prevention Division of Select Agents and Toxins (CDC/DSAT) and U. S. Department of Agriculture, Animal and Plant Health Inspection Service, (USDA/APHIS) National Select Agent Program. The University has been inspected by the CDC/DSAT and USDA/APHIS National Select Agent Program for use of HHS Select Agents and Toxins, Overlap Select Agents and Toxins and USDA Select Agents and Toxins.

Per the requirements of 42 CFR 73 the original certificate of registration was issued October 19, 2004, renewal was granted on April 9, 2007 and again on April 1, 2010 for use of select agents at BioSafety Levels 2, 3, and 4 and Animal BioSafety Levels 3 and 4. Inspection by CDC/DSAT and USDA/APHIS occurred January 9th to 20th 2012, for the current renewal cycle and approval was granted on March 21, 2012 for three years. The University has been registered with Health and Human Services, Centers for Disease Control and Prevention as a select agent facility since 1997. The University has a Responsible Official and four Alternate Responsible Officials.

Attached please find a copy of the University Of Texas Medical Branch certificate of registration of the possession, use and transfer of select agents and toxins. The registration number has been redacted for security purposes. The registration number will be provided at the time of an official CDC/USDA Form 2 transfer of select agents.

Please feel free to contact me should you require additional information.

Sincerely,

A handwritten signature in black ink that reads "Domenica Zimmerman".

Domenica Zimmerman
BioSafety Officer
Alternate Responsible Official
UTMB Select Agent Program

Certificate of Registration

Entity Name: **University of Texas Medical Branch**
 Address: **301 University Boulevard**
Galveston, TX 75555-0633

Registration #: _____
 Effective Date: **March 21, 2012**
 Expiration Date: **March 21, 2015**

USDA

Responsible Official: **Michael Shriner**
 Alternate Responsible Official(s): **Carlos Escobar, Amy Goebel, Scott Weaver, Domenica Zimmerman**

Based on information provided to the CDC Select Agent Program and the APHIS Agricultural Select Agent Programs, the above-named entity is authorized to possess, use, and transfer select agent and toxins under the conditions specified in the entity registration application, in accordance with 42 CFR part 73, 9 CFR part 121, and 7 CFR part 331.

Robbin S. Weyant

Robbin S. Weyant, Director
 Select Agent Program
 Centers for Disease Control and
 Prevention

Freda E. Isaac, DVM

Freda E. Isaac, DVM, Director
 Select Agent Program
 Veterinary Services

Charles L. Divan

Charles L. Divan, Branch Chief
 Select Agent Program
 Plant Protection and Quarantine



Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 154039

Using an Existing Dataset or Resource: No

Delayed Onset Study?: No

Clinical Trial: No

Enrollment Location: Domestic

NIH Defined Phase III Clinical Trial: No

Study Title: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

Planned Enrollment

Comments: This project is not required to enroll subjects. The research has exemption approval and only involves the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens.

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/Not Reported	Female	Male	Unknown/Not Reported	Female	Male	Unknown/Not Reported	
American Indian/Alaska Native	0	0		0	0					0
Asian	0	0		0	0					0
Native Hawaiian or Other Pacific Islander	0	0		0	0					0
Black or African American	0	0		0	0					0
White	0	0		0	0					0
More than One Race	0	0		0	0					0
Unknown or Not Reported										
Total	0	0		0	0					0

Cumulative Enrollment

NOTE: No cumulative inclusion enrollment data exists in the previous inclusion format or modified format. Although prompted to do so, the PD/PI did not enter information in the modified format. No data can be provided.



Grant Number: 5R01AI098775-02

Principal Investigator(s):

Maria Elena Bottazzi
PETER J HOTEZ (contact), PHD
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Leanne Brooks Scott
Business Official
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Budget Period: 05/01/2013 – 04/30/2014

Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$1,085,321 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Michael W. Fato

Additional information follows

SECTION I – AWARD DATA – 5R01AI098775-02**Award Calculation (U.S. Dollars)**

Federal Direct Costs	\$955,528
Federal F&A Costs	\$129,793
Approved Budget	\$1,085,321
Federal Share	\$1,085,321
TOTAL FEDERAL AWARD AMOUNT	\$1,085,321

AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$1,085,321
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SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
2	\$1,085,321	\$1,085,321
3	\$1,134,359	\$1,134,359
4	\$1,165,726	\$1,165,726
5	\$1,165,855	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number: 93.855
EIN: 1741613878A1
Document Number: RAI098775A
Fiscal Year: 2013

IC	CAN	2013	2014	2015	2016
AI	8472315	\$1,085,321	\$1,134,359	\$1,165,726	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC: M51C B / OC: 414E / Released: PII 05/14/2013
Award Processed: 05/15/2013 12:14:44 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI098775-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 5R01AI098775-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Award.
- b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI098775-02

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

This award is issued in accordance with the FY2013 NIH fiscal policies.

RESTRICTION: Under governing PHS Policy, Federal funds administered by the Public Health Service (PHS) shall not be expended for research involving live vertebrate animals without prior approval by the Office of Laboratory Animal Welfare (OLAW) of an Assurance to comply with the PHS Policy on Humane Care and Use of Laboratory Animals and the project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The present award is being made without currently valid verification of IACUC approval for the portion of this project being completed in CHINA with the following restriction: No activities that involve live vertebrate animals may be conducted at Frontier Biosciences located in CHINA pending acceptance by the NIH awarding component of verification of IACUC approval. The Program Officer has approved the funding of this application without the portion of Frontier Biosciences located in CHINA in year 05 as the project is viable without it. No funds may be expended for the foreign site pending the resolution of internal administrative issues. Once these issues have been resolved, this award may be revised to include the study originally planned for the foreign site. Failure to comply with this special condition can result in suspension and/or termination of this award, withholding of support, audit disallowances, and/or other appropriate action.

This award includes funds awarded for consortium activity with NY Blood Center. Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2012/nihgps_ch15.htm#_Toc271265264.

This award includes funds awarded for consortium activity with the University of Texas Medical Branch. Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2012/nihgps_ch15.htm#_Toc271265264.

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at <http://www.selectagents.gov/Regulations.html>) must complete registration with CDC (or APHIS,

depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Vandhana Khurana

Email: khuranav@mail.nih.gov **Phone:** 301-496-7075 **Fax:** 301-493-0597

SPREADSHEET SUMMARY

GRANT NUMBER: 5R01AI098775-02

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	Year 2	Year 3	Year 4	Year 5
TOTAL FEDERAL DC	\$955,528	\$899,886	\$782,695	\$768,645
TOTAL FEDERAL F&A	\$129,793	\$234,473	\$383,031	\$397,210
TOTAL COST	\$1,085,321	\$1,134,359	\$1,165,726	\$1,165,855

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	57.3%	57.3%	57.3%	57.3%
F&A Cost Base 1	\$226,514	\$409,202	\$668,466	\$693,211
F&A Costs 1	\$129,793	\$234,473	\$383,031	\$397,210

A. RPPR COVER PAGE

Project Title: RBD recombinant protein-based SARS vaccine for biodefense	
Grant Number: 5R01AI098775-02	Project/Grant Period: 05/04/2012 - 04/30/2017
Reporting Period: 05/04/2012 - 04/30/2013	Requested Budget Period: 05/01/2013 - 04/30/2014
Report Term Frequency: Annual	Date Submitted: 03/07/2013
Program Director/Principal Investigator Information: PETER J HOTEZ , MD PHD BA Phone number: 832-824-0502 Email: hotez@bcm.edu	Recipient Organization: BAYLOR COLLEGE OF MEDICINE BAYLOR COLLEGE OF MEDICINE 1 BAYLOR PLAZA MS-310 HOUSTON, TX 770303411 DUNS: 051113330 EIN: 1741613878A1 RECIPIENT ID: 35116-N1
Change of Contact PD/PI: N/A	
Administrative Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu	Signing Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu
Human Subjects: Yes HS Exempt: Yes Exemption Number: E4 Phase III Clinical Trial:	Vertebrate Animals: Yes
hESC: No	Inventions/Patents: No

B. ACCOMPLISHMENTS**B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?**

The major goals of the project are: Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate (Timeline Year 1-3). Specific Aim 2: Process development, characterization, formulation and stability profiling (Timeline Year 2-4) and Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation (Timeline Year 4-5).

As proposed, for this reporting period activities related to Specific Aim 1 (Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate) were initiated. Specifically, we have achieved 50% completion of the activities related to the sub-specific aims 1.A. Feasibility of scalable expression, 1.B. Antigenicity and functionality and 1.C. Immunogenicity. For sub-specific aim 1.D. Efficacy, 33.3% of this activity has been completed. The goals will not change for the next reporting period and no significant changes in approach or methods are envisioned.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: RPPR MEB_2_Final PDF.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For Year 2 activities will continue for Specific Aim 1 and new activities under Specific Aim 2 will be initiated.

Briefly for Specific Aim 1, we will continue to evaluate methods to resolve the solubility problem of the bacterial expressed protein, such as performing RBD gene harmonization to increase the ability of bacteria cells to process and translate proteins correctly and to expand the expression refolding strategies to find optimal folding conditions that could lead to a soluble bacteria expressed recombinant RBD protein. In addition, we will continue to further study the yeast expression/fermentation patterns of RBD193-N3, RBD219-N1 and RBD219-N3 and we will extend the initial work to optimize the fermentation and purification of rRBD proteins and improve yield, purity and stability. Immunogenicity and antigenicity will continue to be evaluated in parallel to the production efforts. Detection of the antibody and cellular immune responses induced by rRBD proteins after vaccination in the presence or absence of GLA (Glycopyranosyl lipid A) and/or alum adjuvants will continue followed by the evaluation of cross-neutralizing activity with a SARS-CoV pseudovirus neutralization assay. We have been in close contact with our industrial partner ImmuneDesign. They are providing valuable technical advice and we have executed a Material Transfer Agreement to obtain the necessary adjuvants. These experiments will include the optimization of the immunization regimens, such as adjuvant formulation (GLA-AF, GLA-SE, or alum), antigen doses (1, 5, 10, or 20 µg), and administration number and interval of immunizations. Mouse sera, spleens and lymph nodes will be collected 10 days after last boost for detection of the RBD-specific antibody and T cell immune responses and neutralization as described above. Finally, we will test the protective efficacy of rRBD proteins against lethal challenge with either homologous or heterologous strains of viruses. We will continue to closely coordinate with the consortium partners NYBC and UTMB to fully execute the year 2 studies.

B.2 Year 1 Accomplishments. For this reporting period the major activities were linked to **Specific Aim 1:** Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. Overall yeast expression revealed that at least one of our constructs (rRBD193-N1) shows great promise for production and scale-up (up to 0.5 g/L yields). Briefly, two receptor-binding domain (RBD) gene sequences of the SARS-CoV spike (S) protein, named RBD193 and RBD 219 (Du L et al., *Viral Immunol.* 2010 Apr;23(2):211-9.; He Y et al., *Vaccine* 2006;24:5498–508; Du L et al., *Virology* 2009;393:144-50), were cloned into two protein expression systems, *E. coli* (bacteria) and *Pichia pastoris* (yeast). The bacterial-expressed and yeast-expressed proteins were produced and evaluated based on 1A. Feasibility of scalable expression - yield, scalability, purity and preliminary stability profile, 1B. Antigenicity - recognition of recombinant (r) RBD protein by conformation-dependent monoclonal antibodies (mAbs), 1C. Immunogenicity - ability of the rRBD protein to elicit RBD-specific humoral and cellular immune responses and 1D. Efficacy - obtain and expand various strains of SARS-CoV to be used for challenge studies.

Specific Aim 1.A. Feasibility of scalable expression.

1.A.1. *E. coli* (bacteria) Expression. The wild type (wt) RBD193 and RBD219 gene sequences were codon optimized, cloned and expressed in the pET/BL21 system. Evaluation of the proteins expressed at the shaker flask level showed that high yield expression could be observed but the rRBD protein was insoluble and found in the inclusion bodies (data not shown). To increase the solubility of these bacterial expressed rRBD proteins, we optimized various parameters including: changes in IPTG concentration; different expression temperatures (16-42°C); differences in the induction time (1 hr to overnight); and modified the cell lysis methods. Additional strains of *E. coli* were also evaluated such as BL21-RIPL; Rosetta2 pLysS; and Origami2 pLysS. None of these optimization strategies nor the usage of additional bacterial strains helped increase the solubility of the expressed rRBD protein. Future proposed experiments are described in Section B6 of this report.

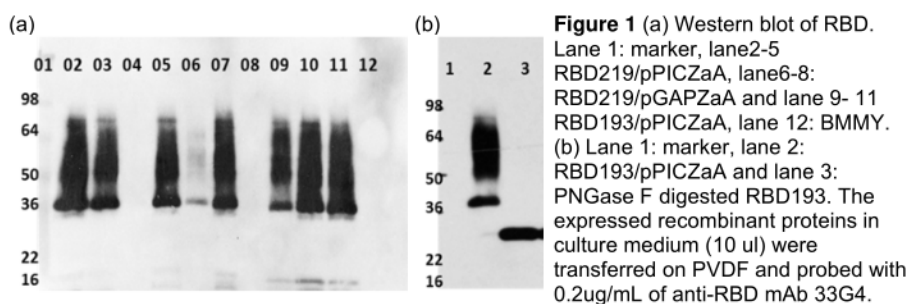
1.A.2. *Pichia pastoris* (yeast) Expression. We evaluated the yeast expression system since it is well proven to overcome production hurdles traditionally observed with the *E. coli* system. Briefly, the wt RBD193 and RBD219 codon optimized genes were engineered and cloned into two yeast expression vectors, pPICZαA and pGAPZαA. Proteins were initially expressed in shaker flasks and results showed good feasibility of soluble rRBD proteins expressed in the pPICZαA system. This system was selected for further evaluation.

1.A.3. Protein yield, purity, and preliminary stability profile.

Protein yield. As stated above, initial shaker flask results showed good predicted protein yields based on visible bands on an SDS-PAGE Coomassie stained gels (data not shown). This meets our internal criteria to proceed and evaluate protein expression in fermenters rather than shaker flask. However, after initial purity and stability evaluation by Western immunoblot analysis results indicate that the wtRBD proteins are either heavily glycosylated or aggregated (Figure 1a); high degree of glycosylation of the RBD protein was confirmed by digesting the protein with N-glycosidase PNGase F (Figure 1b).

This finding could potentially limit our ability to have an accurate and reproducible control during scale-up production and quality control testing. No evidence of aggregation was observed indicating a promising stability profile. In parallel we evaluated the yields expressing the wt RBD193 clone at the 5L fermentation scale; results showed that the wt RBD193 protein during the fermentation process had a toxic effect on the host yeast cell viability and predicted protein yields were not achieved. This finding would impede reaching the required yields needed for large-scale production. Due to this finding we considered a more detailed assessment of the cloning strategy by using gene deletion and mutagenesis strategies (see below).

Preliminary stability profile. To evaluate further the high degree of glycosylation observed and to determine if this post-translational modification could be involved in the toxicity during protein production, the deletion and/or site-directed mutagenesis of the RBD predicted N-linked glycosylation sites was conducted serially. The deletion/mutagenesis produced three different and new inserted RBD genes into the vector pPICZαA for both RBD193 and RBD219: a) N1: removal of 1st glycosylation site; N2: removal of the 1st site and mutation of the 2nd site; and, N3: removal of the 1st site and mutation of the 2nd and 3rd sites. The N1, N2 and N3 yeast-expressed recombinant proteins for RBD193 and RBD219 were evaluated and compared based on expression patterns and yields. Results show that the removal and mutation of the N-linked glycosylation sites facilitated



the production of a soluble macromolecule that can be detected as a distinct and discrete band on a western blot probed with a conformational epitope-specific mAb 33G4 (Figure 2). These data are encouraging because it would allow us to have accurate and reproducible control during scale-up production and quality control testing. However, we also observed that successive de-glycosylation decreased the levels (yield) of expression ($N1 > N2 > N3$). This suggests the necessity to strike a balance between yield and quality control to produce a discrete protein product.

Yield and purity of RBD193N1. A fermentation run at the 5L scale of the clone RBD193N1 showed that the deletion of the sequences encoding for the first N-glycosylation site resolved the issue of toxicity and restored expected yeast cell viability. After the 5L fermentation run a yield of 400 mg/L total expressed recombinant protein was estimated based on densitometry (Figure 3a). Initial attempts to purify rRBD193-N1 from the fermented culture using hydrophobic interaction chromatography and subsequent size exclusion column allowed us to obtain 95% of purified protein evidenced both by SDS-PAGE Coomassie stained gels and Western blot analysis (Figure 3b). These are very promising results and will allow us to continue to focus our work on the yeast expression system for further process development.

1.B. Antigenicity. The rRBD193-N1 was evaluated by Western blot analysis (Figure 4A) with a series of RBD-specific mAbs (He Y et al., Vaccine 2006;24:5498–508; Du L et al., Virology 2009;393:144–50). A mammalian 293T cell-expressing wtRBD193 was used as positive control (He Y et al., J. Immunol. 2005; 174:4908–15). Results show that the rRBD193-N1 protein (5 µg/well) could be recognized by all the tested conformational epitope-specific mAbs and linear epitope-specific mAbs (0.33 µg/ml), with the strongest reaction observed in Conf V mAb 33G4 and the linear mAb 17H9. By ELISA (Figure 4B), results show that rRBD193-N1 (1 µg/ml) could also react with all tested mAbs (3.33 µg/ml), although its reactivity with Conf I mAb 24H8 and Conf VI mAb 19B2 is lower than that of wtRBD193. Both assays show consistent results indicating that rRBD193-N1 protein maintains conformation and antigenicity despite deletion of the N1 glycosylation site.

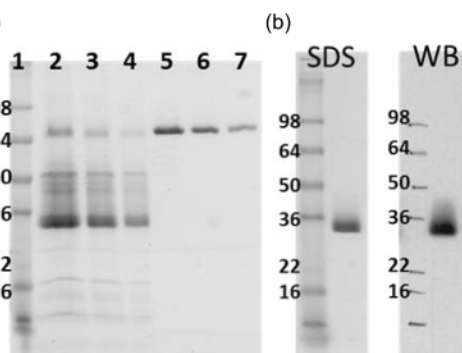
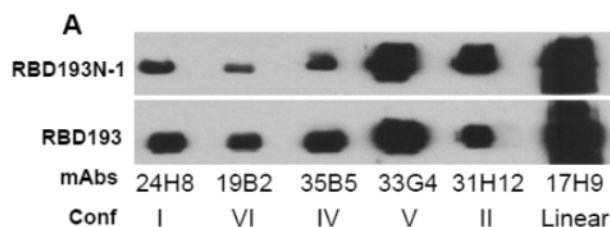


Figure 2. Western blot analysis of recombinant RBDs with different glycosylation sites removed. The expressed RBDs were transferred on PVDF membrane and probed with 0.2µg/mL of anti-RBD mAb 33G4. Lane 1: protein standard. Lane 2 wt-RBD193. Lane 3 RBD193-N1, Lane 4 RBD193-N2, Lane 5 RBD-N3, Lane 6: wtRBD219, Lane: 7 RBD219-N1, Lane 8: RBD219-N2 and Lane 9: RBD219-N3.

Figure 3 (a) SDS PAGE of RBD193-N1 and BSA standard. Lane 1: marker, 10 uL, 5 uL and 2.5 uL of RBD193-N1 in lane 2, 3 and 4 respectively followed by 900ng, 450ng and 225ng of BSA in lane 5, 6 and 7 respectively. **(b)** SDS PAGE (left figure) and western blot (right figure) of 2mg purified RBD 193-N1. Noted that western blot was probed with 0.2µg/mL of anti-RBD mAb 33G4.

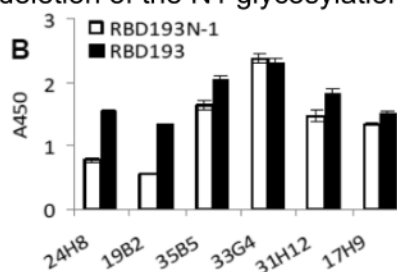


Figure 4. Antigenicity and functionality analysis of purified SARS RBD193N-1 protein. Reactivity of RBD193N-1 protein with mAbs specific for the conformational and linear epitopes of RBD by Western blot (A) and ELISA (B), respectively. Wild-type SARS RBD193 protein was included as the positive control. The data in (B) are expressed as the mean ± standard deviation (SD) of duplicate wells.

1C. Immunogenicity. The rRBD193-N1 protein was used to immunize 6-8 week-old BALB/c mice for three times at 3-week intervals after adsorption to Alhydrogel® adjuvant (InvivoGen, CA). The wtRBD193 (mammalian) protein and PBS were used as positive and placebo controls, respectively. Sera were collected before immunization and 10 days post-each vaccination. Determination of the anti-RBD antibody titers, their neutralizing capability and the isotypes are ongoing.

1D. Efficacy. We have obtained and expanded various strains of SARS-CoV. Clinical or recombinant isolates were generated by reverse genetics, some of which were mouse-adapted (MA). Unlike the transgenic mouse model where lethality of SARS-CoV-infected animals involved severe pathological lesions of both lungs and brains, the wt mice infected by MA virus appear to die of pulmonary pathology with minimal, if any, of CNS involvement. The newly established stocks of MA viruses are ready to test the efficacy of rRBD proteins to protect against heterologous challenge (i.e., cross protection) in wt mice.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, Jones K, Zhan B, Hotez PJ. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. Expert Rev Vaccines. 2012 Dec;11(12):1405-13. PubMed PMID: 23252385; PubMed Central PMCID: PMC3586247.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	HOTEZ, PETER,	PII		BA,PHD, MD	PD/PI	EFFORT	0	0			NA
	Y	JIANG, SHIBO,			PHD,MD	PD/PI		0	0			NA
	N	Tao, Xinrong,			Ph.D.	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0	0			NA
	Y	Zhan, Bin,			MD	Co-Investigator		0	0			NA
	N	Hudspeth, Elissa,			BS	Technician		0	0			NA
	N	Seid, Chris,			Ph.D.	Staff scientist (Doctoral level)		0	0			NA
	N	Chag, Shivali,			MS	Non-Student Research Assistant		0	0			NA
	N	Chen, Wen,			Ph.D.	Non-Student Research Assistant		0	0			NA
	N	Tricoche, Nancy,			BS	Non-Student Research Assistant		0	0			NA
eRA Commons User Name	N	Ma, Cuiqing,			Ph.D	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0	0			NA
	Y	Chan, Tehseng,			MD, PhD	Co-Investigator		0	0			NA
eRA Commons User Name	Y	Du, Lanying,			PHD	Co-Investigator		0	0			NA
	Y	Tseng, Chien-Te,			PHD,MS	Co-Investigator		0	0			NA
	Y	Bottazzi, Maria,			PHD	PD/PI		0	0			NA
	Y	LUSTIGMAN, SARA,			PHD	Co-Investigator		0	0			NA
	N	Nino, Diane,			BSci	Project Manager		0	0			NA

Glossary of acronyms:

S/K - Senior/Key
 DOB - Date of Birth
 Cal - Person Months (Calendar)
 Aca - Person Months (Academic)
 Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation
 SS - Supplement Support
 RE - Reentry Supplement
 DI - Diversity Supplement
 OT - Other
 NA - Not Applicable

D.2 PERSONNEL UPDATES**D.2.a Level of Effort**

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

NOTHING TO REPORT

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: ALL_OtherSupport.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

NOTHING TO REPORT

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

NOTHING TO REPORT

OTHER SUPPORT – HOTEZ, PETER J.**CHANGES:**

32472 – grant ended on 12/31/12

AI 90577 – grant ended on 12/31/12

1016395 – effort increased by grant – new grant was submitted and awarded on 4/20/12
DHHS/Texas A&M contract – was awarded on 11/1/12**CURRENT**23386 Hotez (PI) 01/01/2011-12/31/2014

Sponsor: Dutch Government

€842,857

Title: Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the *Na*-GST-1 and *Na*-APR-1 hookworm antigens in both adults and children.1016395 Hotez (PI) 08/01/2011 - 07/31/2013 Sponsor:

Title: Human Hookworm Vaccine Initiative 3 \$1,491,311

Clinical Development and Evaluation of the *Na*-GST-1 and *Na*-APR-1 Hookworm Vaccine AntigensThe project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*. 04/20/2012 – 03/19/2014 0 cal

\$220,280

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

Sponsor: National Institutes of Health

\$1,277,421

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi (Center Director, Consultant) 11/01/2012 – 12/31/2017

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$329,862

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

Role: Instructor

OVERLAP

None

OTHER SUPPORT - BOTTAZZI, MARIA ELENA**CHANGES:**

32472 – grant ended on 12/31/12

1016395 – effort decreased by EFFORTPrivate Source

– new grant was submitted and awarded on 4/20/12

DHHS/Texas A&M contract – was awarded on 11/1/12

R01AI105431-01 - new grant submitted and awarded on 01/15/2013

ACTIVE

23386 Hotez (PI)

01/01/2011-12/31/2014

EFFORT

Sponsor: Dutch Government

\$285,715

Title: Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the Na-GST-1 and Na-APR-1 hookworm antigens in both adults and children.

Role: Sub-PI

1016395 Hotez (PI)

08/01/2011 - 07/31/2013

EFFORTSponsor: Private Source

Title: Human Hookworm Vaccine Initiative 3 \$1,491,311

Clinical Development and Evaluation of the Na-GST-1 and Na-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Role: Co-Investigator

Hotez (PI)

04/20/2012 – 03/19/2014

0 cal

Private Source

\$220,280

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

Role: Director of Product Development

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

EFFORT

Sponsor: National Institutes of Health

\$1,277,421

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi (Center Director, Consultant)

11/01/2012 – 12/31/2017

EFFORT

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$329,862

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

1R01AI105431-01 Lustigman (PI)

01/15/2013 – 12/31/2017

EFFORT

Sponsor: NIH via New York Blood Center \$270,000

Title: Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Role: Sub-PI

OVERLAP: None

OTHER SUPPORT - ZHAN, BIN**CHANGES:**

32472 – grant ended on 12/31/12

R01A1078314-03 – effort increased by R01A1056189-07 – effort decreased by 1016395 – effort decreased by – new grant was submitted and awarded on 4/20/12

DHHS/Texas A&M contract – was awarded on 11/1/12

R01A1105431-01 - new grant submitted and awarded on 01/15/2013

ACTIVE1 R01A1078314-03 Lustigman (PI) 08/25/2009 – 07/31/2014

NIH/NIAID (Sub-award from New York Blood Center) \$46,017

The development of a recombinant vaccine against human onchocerciasis

The major goal of this subcontract is clone, express, characterize and optimize the expression of the eight selected *Onchocerca* vaccine candidate antigens (rOvAgs) using the yeast *Pichia* eukaryotic system.

Role: Co-PI

2R01A1056189-07 Aroian (PI) 08/01/2010 – 04/30/2014

NIH (sub-award from UCSD) \$18,819

B. thuringiensis Crystal Proteins as Powerful Next-Generation Anthelmintics

The major goal of this subcontract is to test the effects of different formulated Cry5B against hookworm using *Ancylostoma ceylanicum*/hamster model

Role: Co-PI

23386 Hotez (PI) 01/01/2011-12/31/2014

Dutch Government \$285,715

Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the Na-GST-1 and Na-APR-1 hookworm antigens in both adults and children.

Role: Director of Molecular Biology

1016395 Hotez (PI) 08/01/2011 – 07/31/2013

Human Hookworm Vaccine Initiative 3 \$1,491,311

Sponsor:

Title: Clinical Development and Evaluation of the Na-GST-1 and Na-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Role: Director of Molecular Biology

Hotez (PI) 04/20/2012 – 03/19/2014 \$220,280

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

Role: Director of Molecular Biology

R01A1098775-01 Hotez/Bottazzi/Jiang (MPI) 05/01/2012 – 04/30/2017

Sponsor: National Institutes of Health \$1,277,421

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Role: Director of Molecular Biology

Hotez (Center Director, Consultant) 07/01/2012 – 12/31/2017
Sponsor: Department of Health and Human Services / Texas A&M Univ. \$329,862
Title: Centers for Innovation in Advanced Development and Manufacturing
The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.
Role: Instructor

EFFORT

1R01AI105431-01 Lustigman (PI) 04/01/2013 – 03/31/2018
Sponsor: NIH via New York Blood Center \$270,000
Title: Development of a novel adjuvant for vaccine sparring
Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.
Role: Investigator

EFFORT

OVERLAP

None

OTHER SUPPORT

SARA LUSTIGMAN

See below for the change being made:

For OPP1017584 -- got three years continuation funding (from 11/1/2012 – 10/31/2015)

For R01AI105431-01 -- new grant submitted 06/2012 and awarded 01/15/2013

ACTIVE:

- 1. 1R01AI078314-01A2** (PI: S. Lustigman) 8/2009 – 7/2014
NIH/NIAID \$714,313

(b)(6)

The development of a recombinant vaccine against human onchocerciasis

A collaborative research effort focused on the preclinical research and development process that will result, through a robust screening process, with the discovery of the best 2 recombinant *O. volvulus* vaccine antigens with the highest probability for success at inducing protective immunity in humans. The vaccine will target the *O. volvulus* larvae, known to be vulnerable to host immunological attack.

- 2. OPP1017584** (PI: J. McKerrow; Co-PI: S. Lustigman) 11/1/2012 – 10/31/2015
Bill & Melinda Gates Foundation \$304,347 (subcontract)

(b)(6)

Developing a macrofilaricidal drug for onchocerciasis using Anacor's novel oxaborole technology

A collaborative research effort between the University of California San Francisco Sandler Center, Anacor Pharmaceuticals and LFKRI of the NYBC to discover new drug therapies for the treatment of river blindness (onchocerciasis). The collaboration's goal is to identify a novel, potent macrofilaricidal drug candidate that is capable of killing adult worms.

Overlap: none

- 3. R01AI098775-01** MPI: Hotez/Bottazzi/Jiang; Co-PI S. Lustigman 05/01/2012 – 04/30/2017
NIH/NIAID \$300,000 (subcontract)

(b)(6)

RBD Recombinant Protein-based SARS Vaccine for Biodefense

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Overlap: none

- 4. 1R01AI105431-01** (PI: S. Lustigman) 1/2013 – 12/2017
NIH/NIAID \$1,052,327 (including three subcontracts)

(b)(6)

Development of a novel adjuvant for vaccine sparring

Adjuvants are integrated into vaccines to insure their effectiveness and to support antigen sparing. Currently, alum is the only adjuvant licensed in the U.S., but it has had limited effectiveness when used with commercial flu vaccines. Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Overlap: none

E. IMPACT**E.1 Not Applicable****E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?**

NOTHING TO REPORT

E.3 Not Applicable**E.4 WHAT DOLLAR AMOUNT OF THE AWARDS BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?**

NOTHING TO REPORT

F. CHANGES

F.1 Not Applicable for R01
F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM NOTHING TO REPORT
F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS F.3.a Human Subjects No Change
F.3.b Vertebrate Animals No Change
F.3.c Biohazards No Change
F.3.d Select Agents File uploaded: RPPR F3d.pdf

SARS-CoV became a select viral agent as of December 4, 2012. UTMB has been working diligently with various regulatory agents, both on and off the UTMB campus, to comply with all regulations concerning the usage of select agents. In short, we have made an inventory of the SARS-CoV that we have in the laboratory and moved our animal (A).BSL-3 laboratories from Mary Moody Northern (MMN) into the Galveston National Laboratory complex as of December 4th, 2012 with 24/7 security service. During this transition stage our group feels strongly that there will be no negative impact on the ongoing SARS program under this grant and if any, it will be minimal. The biocontainment level for SARS-CoV remains the same, level-3, however, the antigen became classified as a select agent.

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

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 SKMBT_C65413022214530.pdf
 RPPR G1.pdf

G.2 Not Applicable**G.3 Not Applicable****G.4 HUMAN SUBJECTS****G.4.a Does the project involve human subjects?**

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

G.4.c ClinicalTrials.gov**Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?**

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT**Are there personnel on this project who are newly involved in the design or conduct of human subjects research?**

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)**Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?**

No

G.7 VERTEBRATE ANIMALS**Does this project involve vertebrate animals?**

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional	Address
--------------------	------	---------------	---------

		District	
Primary: BAYLOR COLLEGE OF MEDICINE	051113330	TX-007	BAYLOR COLLEGE OF MEDICINE ONE BAYLOR PLAZA HOUSTON TX 770303411
New York Blood Center	073271827	NY-014	310 East 67 Street New York NY 100656275
The University of Texas Medical Branch	800771149	TX-014	301 University Boulevard Galveston TX 775550156
Texas Childrens Hospital	074615394	TX-007	1102 Bates Street Houston TX 770302399

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No



Environmental Health & Safety
Biological & Chemical Safety Program
Materials Management Building, 2.112
301 University Blvd.
Galveston, Texas 77555-1111
O 409.772.1781 F 409.772.8921

February 11, 2013

To Whom It May Concern

The University of Texas Medical Branch at Galveston (UTMB) is a select agent registered entity with the U.S. Health and Human Services, Centers for Disease Control and Prevention Division of Select Agents and Toxins (CDC/DSAT) and U. S. Department of Agriculture, Animal and Plant Health Inspection Service, (USDA/APHIS) National Select Agent Program. The University has been inspected by the CDC/DSAT and USDA/APHIS National Select Agent Program for use of HHS Select Agents and Toxins, Overlap Select Agents and Toxins and USDA Select Agents and Toxins.

Per the requirements of 42 CFR 73 the original certificate of registration was issued October 19, 2004, renewal was granted on April 9, 2007 and again on April 1, 2010 for use of select agents at BioSafety Levels 2, 3, and 4 and Animal BioSafety Levels 3 and 4. Inspection by CDC/DSAT and USDA/APHIS occurred January 9th to 20th 2012, for the current renewal cycle and approval was granted on March 21, 2012 for three years. The University has been registered with Health and Human Services, Centers for Disease Control and Prevention as a select agent facility since 1997. The University has a Responsible Official and four Alternate Responsible Officials.

Attached please find a copy of the University Of Texas Medical Branch certificate of registration of the possession, use and transfer of select agents and toxins. The registration number has been redacted for security purposes. The registration number will be provided at the time of an official CDC/USDA Form 2 transfer of select agents.

Please feel free to contact me should you require additional information.

Sincerely,

A handwritten signature in black ink that reads "Domenica Zimmerman". The signature is fluid and cursive, with a long, sweeping underline.

Domenica Zimmerman
BioSafety Officer
Alternate Responsible Official
UTMB Select Agent Program

Certificate of Registration

Entity Name: **University of Texas Medical Branch**
 Address: **301 University Boulevard**
Galveston, TX 75555-0633

Registration #: **March 21, 2012**
 Effective Date: **March 21, 2015**
 Expiration Date:

USDA

Responsible Official: **Michael Shriner**

Alternate Responsible Official(s): **Carlos Escobar, Amy Goebel, Scott Weaver, Domenica Zimmerman**

Based on information provided to the CDC Select Agent Program and the APHIS Agent Select Agent Programs, the above-named entity is authorized to possess, use, and transfer select agent and toxins under the conditions specified in the entity registration application, in accordance with 42 CFR part 73, 9 CFR part 121, and 7 CFR part 331.

Robbin S. Weyant

Robbin S. Weyant, Director
 Select Agent Program
 Centers for Disease Control and
 Prevention

Freda E. Isaac, DVM

Freda E. Isaac, DVM, Director
 Select Agent Program
 Veterinary Services

Charles L. Divan

Charles L. Divan, Branch Chief
 Select Agent Program
 Plant Protection and Quarantine



At Baylor College of Medicine (BCM) and New York Blood Center (NYBC) no research is conducted or is planned to be performed under this grant with a Highly Pathogenic Agent or Select Agent. The institutional IBC officials have determined that the work being planned or performed under this grant at these institutions may be conducted at a biocontainment safety level that is lower than BSL3.

At University of Texas Medical Branch (UTMB) the work will involve Select Agents and/or Highly Pathogenic Agents. No changes have been done in the use of the Agent, the type of experiments or required biocontainment level. UTMB documentation of the registration status is included.

Inclusion Enrollment Report Table

This report format should NOT be used for data collection from study participants.

Study Title: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

Total Enrollment: 0

Protocol Number:

Grant Number: R01AI098775-02

PART A. TOTAL ENROLLMENT REPORT : Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
Hispanic or Latino	0	0	0	0
Not Hispanic or Latino	0	0	0	0
Unknown (Individuals not reporting ethnicity)	0	0	0	0
Ethnic Category:Total of All Subjects	0	0	0	0
Racial Categories				
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian Or Other Pacific Islander	0	0	0	0
Black Or African American	0	0	0	0
White	0	0	0	0
More than one race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of All Subjects	0	0	0	0
PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian Or Other Pacific Islander	0	0	0	0
Black Or African American	0	0	0	0
White	0	0	0	0
More than one race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics Or Latinos	0	0	0	0

Inclusion Enrollment Comments: This project is not required to enroll subjects. The research has exemption approval and only involves the collection or study of existing data, documents, records, pathological specimens or diagnostic specimens.



Grant Number: 5R01AI098775-03
FAIN: R01AI098775

Principal Investigator(s):
Maria Elena Bottazzi
PETER J HOTEZ (contact), PHD
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Leanne Brooks Scott
Business Official
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Budget Period: 05/01/2014 – 04/30/2015
Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$1,134,359 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Michael W. Fato
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI098775-03**Award Calculation (U.S. Dollars)**

Federal Direct Costs	\$899,886
Federal F&A Costs	\$234,473
Approved Budget	\$1,134,359
Federal Share	\$1,134,359
TOTAL FEDERAL AWARD AMOUNT	\$1,134,359

AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$1,134,359
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SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
3	\$1,134,359	\$1,134,359
4	\$1,165,726	\$1,165,726
5	\$1,165,855	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1741613878A1
Document Number:	RAI098775A
PMS Account Type:	G (Pooled)
Fiscal Year:	2014

IC	CAN	2014	2015	2016
AI	8472315	\$1,134,359	\$1,165,726	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC: M51C B / OC: 414E / Released: P11 04/11/2014
Award Processed: 12/26/2013 10:57:56 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI098775-03

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 5R01AI098775-03

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Award.
- b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm> for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI098775. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI098775-03

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

RESTRICTION: Under governing PHS Policy, Federal funds administered by the Public Health Service (PHS) shall not be expended for research involving live vertebrate animals without prior approval by the Office of Laboratory Animal Welfare (OLAW) of an Assurance to comply with the PHS Policy on Humane Care and Use of Laboratory Animals and the project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The present award is being made without currently valid verification of IACUC approval for the portion of this project being completed in CHINA with the following restriction: No activities that involve live vertebrate animals may be conducted at Frontier Biosciences located in CHINA pending acceptance by the NIH awarding component of verification of IACUC approval. The Program Officer has approved the funding of this application without the portion of Frontier Biosciences located in CHINA in year 05 as the project is viable without it. No funds may be expended for the foreign site pending the resolution of internal administrative issues. Once these issues have been resolved, this award may be revised to include the study originally planned for the foreign site. Failure to comply with this special condition can result in suspension and/or termination of this award, withholding of support, audit disallowances, and/or other appropriate action.

This award includes funds awarded for consortium activity with NY Blood Center and the University of Texas Medical Branch. Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch15.htm#_Toc271265264.

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at <http://www.selectagents.gov/Regulations.html>) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (<http://www.cdc.gov/OD/ohs/biosfty/bmb15/bmb15toc.htm>). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Jason A. Lundgren

Email: lundgrenj@mail.nih.gov **Phone:** 301-594-6355 **Fax:** 301 493 0597

Program Official: Erik J. Stemmy

Email: erik.stemmy@nih.gov **Phone:** (301)-402-3947

SPREADSHEET SUMMARY

GRANT NUMBER: 5R01AI098775-03

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	Year 3	Year 4	Year 5
TOTAL FEDERAL DC	\$899,886	\$782,695	\$768,645
TOTAL FEDERAL F&A	\$234,473	\$383,031	\$397,210
TOTAL COST	\$1,134,359	\$1,165,726	\$1,165,855

Facilities and Administrative Costs	Year 3	Year 4	Year 5
F&A Cost Rate 1	57.3%	57.3%	57.3%
F&A Cost Base 1	\$409,202	\$668,466	\$693,211
F&A Costs 1	\$234,473	\$383,031	\$397,210

A. COVER PAGE

Project Title: RBD recombinant protein-based SARS vaccine for biodefense	
Grant Number: 5R01AI098775-03	Project/Grant Period: 05/04/2012 - 04/30/2017
Reporting Period: 05/01/2013 - 04/30/2014	Requested Budget Period: 05/01/2014 - 04/30/2015
Report Term Frequency: Annual	Date Submitted: 03/14/2014
Program Director/Principal Investigator Information: PETER J HOTEZ , MD PHD BA Phone number: 832-824-0502 Email: hotez@bcm.edu	Recipient Organization: BAYLOR COLLEGE OF MEDICINE BAYLOR COLLEGE OF MEDICINE 1 BAYLOR PLAZA MS-310 HOUSTON, TX 770303411 DUNS: 051113330 EIN: 1741613878A1 RECIPIENT ID: 35116-N2
Change of Contact PD/PI: N/A	
Administrative Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu	Signing Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu
Human Subjects: Yes HS Exempt: Yes Exemption Number: E4 Phase III Clinical Trial:	Vertebrate Animals: Yes
hESC: No	Inventions/Patents: Yes If yes, previously reported: Yes

B. ACCOMPLISHMENTS**B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?**

The major goals of the project are: Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate (Timeline Year 1-3). Specific Aim 2: Process development, characterization, formulation and stability profiling (Timeline Year 2-4) and Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation (Timeline Year 4-5).

As proposed, for this reporting period activities related to Specific Aim 1 (Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate) were initiated. Specifically, we have achieved 50% completion of the activities related to the sub-specific aims 1.A. Feasibility of scalable expression, 1.B. Antigenicity and functionality and 1.C. Immunogenicity. For sub-specific aim 1.D. Efficacy, 33.3% of this activity has been completed. The goals will not change for the next reporting period and no significant changes in approach or methods are envisioned.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: SARS NIH Annual report 2-page summary 03-13-14.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

Yes

Revision/ Supplements #	Revision/ Supplements Title	Specific Aims	Accomplishments
3R01AI098775-02S1	RBD recombinant protein-based SARS vaccine for biodefense	The project plan was to instruct the student on fundamental techniques in molecular biology and biochemistry and provide a broad educational overview on the key steps in developing a vaccine to prevent a major public health threat. The project plan included regular meetings with the research team; one-on-one mentorship meetings to provide feedback on weekly activities; answer questions and provide additional training if needed; and training in various general aspects of research. The student's work fell under Specific Aim 2 of the parent grant, specifically, Process development, characterization, formulation and stability profiling.	The student assisted with process development of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV), a major part of the Specific Aim 2. The student assisted with the discovery of possible purification parameters as well as the identification of in-process samples over the chromatographic purification procedure thereby contributing to Milestone 1: A suitable expression is selected for expression of rRBD in small scale and Milestone 4: Established a reproducible 10L scale process for a stable rRBD-based vaccine in preparation for future technology transfer to a cGMP manufacturing facility.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

ASM Biodefense and Emerging Diseases conference attended and presentation of a poster titled Process development of a SARS vaccine candidate: a yeast-expressed receptor-binding domain of the SARS-CoV spike protein by Wen-Hsiang Chen, Ph.D on 1/27-29, 2014.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For Year 3, activities will continue toward the completion of Specific Aim 1. Using the selected yeast-expressed, rRBD-based SARS candidate vaccine, additional detection of the antibody and cellular immune responses induced by rRBD proteins after vaccination in the presence or absence of GLA (Glycopyranosyl lipid A) and/or alum adjuvants will be explored followed by the evaluation of cross-neutralizing activity with a SARS-CoV pseudovirus neutralization assay.

Our initial studies will assist us in optimizing the immunization regimens for the selected SARS candidate vaccine, including immunization routes, adjuvant formulation, rRBD protein doses, as well as immunization dosages and intervals, in a hope to select the best immunization regimens for subsequent long-term immunogenicity evaluation and SARS-CoV challenge study.

To optimize immunization routes and adjuvant formulation, we will immunize mice via subcutaneous (s.c.) or intramuscular (i.m.) route, respectively, with the selected rRBD protein, in the presence or absence of alum and/or GLA adjuvant, followed by boosting of the mice twice at 3-week intervals. To optimize antigen doses, we will immunize mice with rRBD protein at 1, 5, 10, (or 20 µg if needed), respectively, using the optimized adjuvant and optimal route, and boost once or twice (with 1, 5, or 10 µg rRBD, respectively) at 3-week intervals. The optimization of immunization dosages and intervals will be tested in mice vaccinated with the selected rRBD protein and adjuvant using the optimized adjuvant formulation, optimized rRBD dose and optimal route, followed by boosting of the mice once at 2- or 8-week intervals or not boosted.

Vaccine-induced, RBD-specific immunogenicity, particularly IgG and neutralizing antibodies, will be assessed in vaccinated mouse sera, and T cellular immune responses will be detected using mouse spleens and lymph nodes collected 10 days after last boost. In addition, the ability of the SARS-CoV RBD vaccines to induce cross-neutralizing antibody responses will also be evaluated using SARS pseudovirus and live virus neutralization assays in collected sera of mice vaccinated with the rRBD candidate protein.

The rRBD-induced long-term immune responses will be tested in mice vaccinated with optimized vaccination regimen, and observed for a period of 6 or 12 month, respectively, followed by detection of antibody response, neutralizing activity against SARS pseudovirus and T cell responses as described above.

Once the best vaccination strategy has been established, we will conduct experiments to fully determine the protective efficacy of selected rRBD-based vaccine candidate(s) against lethal SARS-CoV infection. While the immunogenicity and the ability of tested vaccines to restrict viral replication and pathology in the lungs will be used as criteria for the assessment, whether vaccination would result in a Th2-type disease enhancement will be particularly emphasized.

For Year 3, activities we will continue for Specific Aim 2. We will continue optimizing the fermentation conditions and purification scheme for the yeast expressed RBD219-N1. In addition, we will further evaluate the assays which establish identity, yields, purity, conformation and integrity for our target protein after expression (e.g., HPLC-SEC, HPLC-RP, Mass Spectrometry etc.). Also, we will initiate the study for formulation and stabilization of RBD219-N1 in order to produce a formulation of a recombinant vaccine of maximal stability potentially suitable for emergency stockpiling.

We will continue to closely coordinate with the consortium partners NYBC and UTMB to fully execute the year 3 studies.

B.2 Year 2 Accomplishments. Year 2 was a breakout year for our SARS Vaccine development program. We determined that a specific receptor binding domain (RBD) construct known as RBD219-N1 could be expressed in the yeast *Pichia pastoris* at high yield and purity. Moreover, RBD219-N1 was shown to elicit high titers of neutralizing antibodies. Based on our results and following our Year 2 program review RBD219-N1 will be selected for process development and cGMP manufacture.

For this reporting period the major activities performed were linked to Aim 1 and Aim 2. **Aim 1.A. Feasibility of scalable expression.** **1.A.1. *E. coli* (bacteria) Expression.** We completed the evaluation of alternative methods to address the solubility of bacterially expressed proteins. None of the strategies used led to suitable results as compared to the yeast expression strategy (see below) This sub aim has been completed and closed out. **1.A.2. *Pichia pastoris* (yeast) Expression.** The yeast system was selected as the expression platform of choice. Four rRBDs (RBD193-N1, RBD193-N3, RBD219-WT and RBD219-N1) were evaluated for yield (>25mg/L), ease of purification and purity (>90%), antigenicity and immunogenicity. Table 1 ranks the rRBD candidates indicating that RBD219-N1 is the top candidate to advance into process development (Aim 2). To improve fermentation yield (~45mg/L clone OR), additional screening was performed to identify a final research seed stock. More than 80 clones were screened in the presence of different concentrations of Zeocin identifying high copy transformants. Western blot results revealed Clone#27 as the highest expressor

Table 1. Comparison of the four yeast expressed RBD proteins. Note that RBD193-WT was the positive control expressed in mammalian cell 293T (Du et al., Viral Immunol. 2010).

Construct	Fermentation Yield (mg/L)	Antigenicity response (Fig. 3)	IgG Titers (Fig. 4A)	Neutralizing Antibody (Pseudovirus)	Neutralizing Antibody (Live virus)
RBD193-WT	N/A	Medium	3.5×10^5	4×10^4	2.3×10^3
RBD193-N1	~100	Medium	1.8×10^5	4.3×10^3	2.5×10^2
RBD193-N3	<16	Medium	1.9×10^5	1.4×10^4	1.6×10^3
RBD219-WT	~40	High	1.8×10^5	4×10^4	2.2×10^3
RBD219-N1	~45	High	1.4×10^6	4×10^4	4.5×10^3

by SDS-PAGE Coomassie stained gels and Western blot (Figure 1). **Preliminary stability profile.** The rRBD219-N1 soluble purified protein can be detected as a distinct and discrete band on a western blot probed with a conformational epitope-specific mAb 33G4 and with no evidence of degradation or aggregation (data not shown).

Aim 1.B. Antigenicity and functionality. To validate the antigenicity of purified RBD proteins, we performed ELISA with five conformational anti-RBD mAbs (24H8, 19B2, 35B5, 33G4, and 31H12) and one linear anti-RBD mAb (17H9). We found that RBD219-WT and RBD219-N1 (1 µg/mL) exhibited the strongest binding to all conformational mAbs tested at concentration as low as 0.25 µg/mL, although their reactivity to the linear anti-RBD mAb 17H9 was significantly decreased (Figure 2), suggesting that deglycosylated RBD219-N1 protein, like RBD219-WT, was able to maintain conformation and antigenicity, despite the deletion of the N1 glycosylation site. The functionality was also evaluated by detecting the binding ability of these proteins to SARS-CoV's receptor ACE2. The results show that all purified RBD proteins react strongly with either cell-associated or soluble receptor when tested using the ACE2- or RBD-specific mAbs. (Figure not shown.) These results indicated that all RBD proteins with or without mutations maintain functionality. **Aim 1.C. Immunogenicity.** Immunogenicity was evaluated in an established mouse model by immunizing with purified RBD proteins adsorbed to Alum. Antibody responses and neutralizing antibodies were evaluated. Results show that RBD219-N1 induced significantly higher IgG titers against RBD219-WT as compared to all the other RBD proteins. The control RBD193-WT also induced significantly higher IgG titers against RBD219-WT (Figure 3A). For neutralizing antibodies, we tested sera from vaccinated mice 10 days post-last vaccination using both pseudovirus and live SARS-CoV-based neutralization assays. Immunization with RBD219-N1 resulted in significantly higher titers of neutralizing antibodies against live SARS-CoV infection compared to those elicited by the control RBD193-WT, RBD193-N1, RBD193-N3, or RBD219-WT (Figure 3B). In addition, immunization with the control RBD193-WT, RBD219-WT or

(compared to clone OR). Clone#27 was tested for expression yield in a 5L-scale fermentation with a yield of 70.5 mg/L.

1.A.3. RBD219-N1 Protein yield, purity and preliminary stability profile. **Protein yield.** Total fermentation supernatant of clone OR was purified using hydrophobic interaction chromatography and subsequent size exclusion column allowing us to obtain 95% of purified protein evidenced both

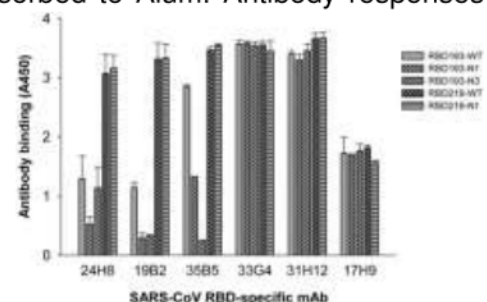
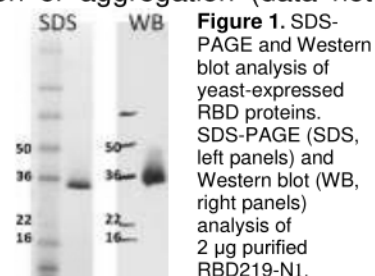


Figure 2. Antigenicity evaluation of SARS-CoV RBD proteins with 0.25 µg/mL anti-RBD mAbs

RBD219-N1 uniformly elicited potent neutralizing antibody responses against SARS pseudovirus infection, being significantly stronger than those induced by the other two RBD proteins (data not shown). As expected, the Alum PBS control did not elicit neutralizing antibody response against both pseudovirus and live SARS-CoV (Figure 3). These data confirm that RBD219-N1 possesses the strongest immunogenicity among RBD proteins tested. **Aim 1.D. Efficacy.** Pilot study

comparing safety, immunogenicity, and efficacy of RBD- and S-based Alum vaccines (Tseng et al., 2010. PLoS One) in a lethal mouse model of SARS was done. Figure 4A shows mice immunized with RBD-219N1 elicited the highest titer of neutralizing antibody and were protected from clinical disease (i.e., weight loss) with no death (Figure 4B and 4C), when compared to those immunized with other vaccines. None of the mice given TBS/alum produced detectable neutralizing antibody whereas their geometric means of lung virus titers were $10^{9.9}$ and $10^{8.9}$ TCID₅₀/g on days 1 and 2 post infection (PI), respectively. In contrast, all vaccinated groups exhibited lower or even undetectable viral titers in the lungs at days 1 and 2 PI (Figure 4D and 4E). Whether mice vaccinated with RBD proteins might exhibit a T_H2-type immunopathology with prominent eosinophil infiltration as those vaccinated with SARS-CoV S protein is currently under investigation, they were more effective in reducing SARS-CoV infection and diseases. **Specific Aim 2.A. Development and optimization of a 10 L scale process.** **2.A.1 Upstream process optimization.** Fermentation conditions including (temperatures, pHs, sorbitol co-feeds, medium salt concentrations, detergents and methanol feed rates) were optimized at 5L scale. The best induction condition was identified to be using a low salt buffer media, at 24°C, and pH 6.5 with a methanol flow rate increased from 11 to 15 mL/L/hr. This condition yield 70.5 mg/L of RBD219-N1 in the fermentation supernatant. **2.A.2 Downstream process.** We purified the RBD219-N1 using a two-step purification process: Butyl Hydrophobic Interaction Chromatography (HIC) followed by Size Exclusion

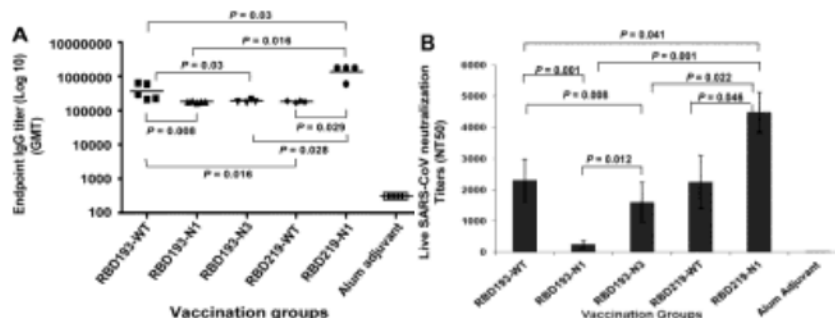


Figure 3. Immunogenicity of RBD proteins. A, IgG antibody by ELISA. B, neutralization antibody detection in mouse sera. The RBD proteins were used to immunize 6-8 week-old BALB/c mice for three times at 3-week intervals after adsorption to alhydrogel adjuvant.

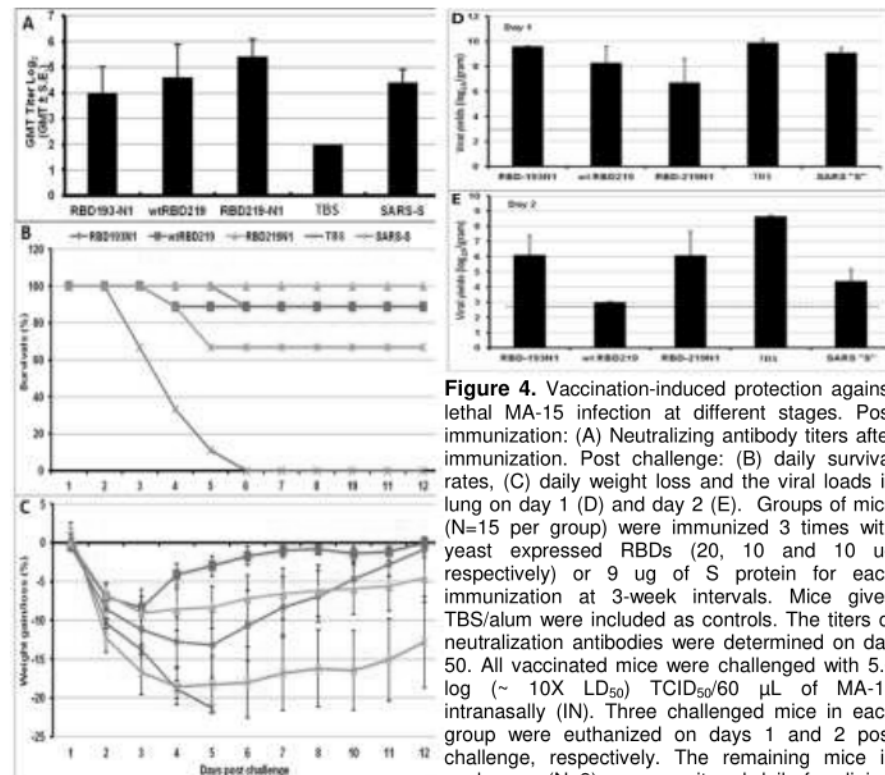


Figure 4. Vaccination-induced protection against lethal MA-15 infection at different stages. Post immunization: (A) Neutralizing antibody titers after immunization. Post challenge: (B) daily survival rates, (C) daily weight loss and the viral loads in lung on day 1 (D) and day 2 (E). Groups of mice (N=15 per group) were immunized 3 times with yeast expressed RBDs (20, 10 and 10 ug respectively) or 9 ug of S protein for each immunization at 3-week intervals. Mice given TBS/alum were included as controls. The titers of neutralization antibodies were determined on day 50. All vaccinated mice were challenged with 5.6 log (~ 10X LD₅₀) TCID₅₀/60 µL of MA-15 intranasally (IN). Three challenged mice in each group were euthanized on days 1 and 2 post challenge, respectively. The remaining mice in each group (N=9) were monitored daily for clinical manifestations (e.g., weight loss), and mortality

mentioned above. The three successive process development runs are scheduled after the optimization of the process is complete.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

Funds were received for a minority supplement on the development and manufacture of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV). The project served as a basis for engaging an under-represented minority high school student in an eight-week long mentored program of biotechnology and biochemistry research. The program was offered in association with the Office of Diversity and Community Outreach at Baylor College of Medicine.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, Jones K, Zhan B, Hotez PJ. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. Expert Rev Vaccines. 2012 Dec;11(12):1405-13. PubMed PMID: 23252385; PubMed Central PMCID: PMC3586247.
PMC Journal - In process	Chen WH, Du L, Chag SM, Ma C, Tricoche N, Tao X, Seid CA, Hudspeth EM, Lustigman S, Tseng CT, Bottazzi ME, Hotez PJ, Zhan B, Jiang S. Yeast-expressed recombinant protein of the receptor-binding domain in SARS-CoV spike protein with deglycosylated forms as a SARS vaccine candidate. Hum Vaccin Immunother. 2013 Dec 30;10(3)PubMed PMID: 24355931.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	HOTEZ, PETER,	PII		BA,PHD, MD	PD/PI	EFFORT	0	0			NA
	Y	Bottazzi, Maria,				PD/PI		0	0			NA
	Y	JIANG, SHIBO,			PHD,MD	PD/PI		0	0	Fudan University	CHINA	NA
	Y	LUSTIGMAN, SARA,			PHD	Co-Investigator		0	0			NA
	N	Hudspeth, Elissa,			BS	Technician		0	0			NA
	N	Zhang, Naru,			Ph.D.	Research Fellow		0	0			NA
	N	Chan, Tehseng,			MD, PhD	Co-Investigator		0	0			NA
	N	Tricoche, Nancy,			BS	Non-Student Research Assistant		0	0			NA
	Y	Tseng, Chien-Te,			PHD,MS	Co-Investigator		0	0			NA
	N	Chag, Shivali,			MS	Non-Student Research Assistant		0	0			NA
	N	Seid, Chris,			Ph.D.	Staff scientist (Doctoral level)		0	0			NA
	N	Chen, Wen,			Ph.D.	Non-Student Research Assistant		0	0			NA
	N	Nino, Diane,			BSci	Project Manager		0	0			NA
	N	Nelson, Frederick,				High School Student		0	2			DS
	N	Pollet, Jeroen,			Ph.D.	Director, Formulation		0	0			NA
	Y	Du, Lanying,			PHD	Co-Investigator		0	0			NA
eRA Commons User Name	N	Tao, Xinrong,			Ph.D.	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0	0			NA

Glossary of acronyms:

S/K - Senior/Key
 DOB - Date of Birth
 Cal - Person Months (Calendar)
 Aca - Person Months (Academic)
 Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation
 SS - Supplement Support
 RE - Reentry Supplement
 DI - Diversity Supplement
 OT - Other
 NA - Not Applicable

D.2 PERSONNEL UPDATES**D.2.a Level of Effort**

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

NOTHING TO REPORT

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: all-OS.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

NOTHING TO REPORT

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

NOTHING TO REPORT

OTHER SUPPORT – HOTEZ, PETER J.**CHANGES:**

Private Source grant: received funding for another two years until 4/19/16

Newly awarded:

Two grants from Private Source subaward

University of Malaya

Private Source

Instituto Carlos Slim de la Salud

CURRENT

23386 Hotez (PI) 01/01/2011-12/31/202014

Sponsor: Dutch Government

€842,857

Title: Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the *Na*-GST-1 and *Na*-APR-1 hookworm antigens in both adults and children.

EFFORT

1016395 Hotez (PI) 08/01/2011 - 04/30/2015

Sponsor: Private Source

Title: Human Hookworm Vaccine Initiative 3 \$871,809

Clinical Development and Evaluation of the *Na*-GST-1 and *Na*-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

EFFORT

Hotez (PI) 04/20/2012 – 04/19/2016

Private Source

\$225,928

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

EFFORT

5R01AI098775-02 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

Sponsor: National Institutes of Health

\$955,528

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

EFFORT

Bottazzi (Center Director, Consultant) 11/01/2012 – 12/31/2017

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$255,928

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

Role: Instructor

EFFORT

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017

Sponsor: Private Source

\$328,435

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

EFFORT

Hotez (PI) 01/01/2014 – 12/31/2016

Sponsor: University of Malaya

\$250,000

Title: Malaysian Neglected Tropical Disease Initiative

Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

EFFORT

Hotez (PI)	01/01/2014 – 12/31/2017		EFFORT
Sponsor:	Private Source	\$160,000	
Title: West Nile Virus vaccine development			
Main goal is to support West Nile Virus vaccine development.			
Hotez/Bottazzi (MPI)	01/01/2014 – 12/31/2017		EFFORT
Sponsor:	Private Source	\$179,348	
Title: Hookworm Vaccine Discovery Program			
The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.			
Bottazzi/Hotez (MPI)	07/01/2012 – 12/31/2014		EFFORT
Sponsor:	Instituto Carlos Slim de la Salud	\$709,333	
Title: Slim Initiative for Antipoverty Vaccine Development			
The main goal of this project is to build a new generation of urgently needed vaccines for the neglected diseases, and to build capacity for vaccine development in Mexico.			

OVERLAP

None. If funded, appropriate adjustments will be made to ensure that Dr. Hotez's time will total no than 100% on active projects at any given time

OTHER SUPPORT - BOTTAZZI, MARIA ELENA**CHANGES:**

23386 – grant ended on 12/31/13

Private Source grant: received funding for another two years until 4/19/16

Newly awarded:

Two grants from Private Source

European Union via AIGHD subaward

University of Malaya

Private Source

Instituto Carlos Slim de la Salud

ACTIVE

1016395 Hotez (PI) 08/01/2011 – 04/30/15

EFFORT

Sponsor: Private Source

Title: Human Hookworm Vaccine Initiative 3 \$871,809

Clinical Development and Evaluation of the Na-GST-1 and Na-APR-1 Hookworm Vaccine Antigens

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Role: Co-Investigator

Hotez (PI) 04/20/2012 – 04/19/2016

EFFORT

Private Source

\$225,928

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

Role: Director of Product Development

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

EFFORT

Sponsor: National Institutes of Health \$955,528

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi (Center Director, Consultant) 11/01/2012 – 12/31/2017

EFFORT

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$255,928

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

1R01AI105431-01 Lustigman (PI) 01/15/2013 – 12/31/2017

EFFORT

Sponsor: NIH via New York Blood Center \$177,000

Title: Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Role: Sub-PI

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017

EFFORT

Sponsor: Private Source

\$328,435

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

HOOKVAC Bottazzi (PI)

10/1/2013 – 9/30/2017

EFFORT

RPPR

Sponsor: European Union via sub from (AIGHD) \$91,121

Title: Developing and Testing a novel, low-cost, effective HOOKworm VACcine to Control Human Hookworm Infection in endemic countries

Major goals of the project are to perform technology transfer of processes for fermentation purification and analytical testing of the human hookworm vaccine.

Hotez (PI) 01/01/2014 – 12/31/2016

EFFORT

Sponsor: University of Malaya \$250,000

Title: Malaysian Neglected Tropical Disease Initiative

Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

Role: Co-I

Hotez (PI) 01/01/2014 – 12/31/2017

EFFORT

Sponsor: Private Source \$160,000

Title: West Nile Virus vaccine development

Main goal is to support West Nile Virus vaccine development.

Role: Co-I

Hotez/Bottazzi (MPI) 01/01/2014 – 12/31/2017

EFFORT

Sponsor: Private Source \$179,348

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Bottazzi/Hotez (MPI) 07/01/2012 – 12/31/2014

EFFORT

Sponsor: Instituto Carlos Slim de la Salud \$709,333

Title: Slim Initiative for Antipoverty Vaccine Development

The main goal of this project is to build a new generation of urgently needed vaccines for the neglected diseases, and to build capacity for vaccine development in Mexico.

OVERLAP

None. If funded, appropriate adjustments will be made to ensure that Dr. Bottazzi's time will total no than 100% on active projects at any given time

Other Support

Dr. Lanying Du received an R21 grant (R21 AI109094) for identification of critical neutralizing domain-based vaccines against new SARS-like virus MERS-CoV. She will put EFFORT of her effort for the R21 project (as shown below), but keep her originally approved EFFORT effort unchanged.

Dr. Sara Lustigman changed her Other Support for NIH/NIADI R01 grant (1R01AI078314-01A2) and a Private Source Private Source (OPP1017584) from EFFORT to EFFORT respectively. She also received several new grants from Private Source (OPP1086618, OPP1099849, OPP1083910, with effort of EFFORT EFFORT and EFFORT respectively), and NIH/NIAD (1R56 1AI101372-01A1, with effort of EFFORT). She will keep her efforts on other grants unchanged.

LANYING DU

ACTIVE

1R21AI109094-01 (Du)
NIH/NIAD

12/15/2013 – 11/30/2015

EFFORT

Critical neutralizing domain-based vaccines against new SARS-like virus hCoV-EMC

The major goal of this project is develop a critical neutralizing domain (CND)-based subunit vaccine for the prevention of the newly identified MERS-CoV or other coronaviruses that may cause future outbreaks in humans.

Role: PI

OVERLAP: None

SHIBO JIANG

ACTIVE

1R21AI109094-01 (Du)
NIH/NIAD

12/15/2013 – 11/30/2015

EFFORT

Critical neutralizing domain-based vaccines against new SARS-like virus hCoV-EMC

The major goal of this project is develop a critical neutralizing domain (CND)-based subunit vaccine for the prevention of the newly identified MERS-CoV or other coronaviruses that may cause future outbreaks in humans.

Role: Co-Investigator

OVERLAP: None

SARA LUSTIGMAN
ACTIVE**1. 1R01AI078314-01A2** (PI: S. Lustigman) 8/2009 – 7/2014

(b)(6)

NIH/NIAID

\$603,776

The development of a recombinant vaccine against human onchocerciasis

A collaborative research effort focused on the preclinical research and development process that will result, through a robust screening process, with the discovery of the best 2 recombinant *O. volvulus* vaccine antigens with the highest probability for success at inducing protective immunity in humans. The vaccine will target the *O. volvulus* larvae, known to be vulnerable to host immunological attack.

Overlap: none

2. OPP1017584 (Bill & Melinda Gates Foundation) (PI: J. McKerrow; Co-PI: S. Lustigman)

\$304,347 (subcontract)

11/1/2012 – 10/31/2015

(b)(6)

Developing a macrofilaricidal drug for onchocerciasis using Anacor's novel oxaborole technology

A collaborative research effort between the University of California San Francisco Sandler Center, Anacor Pharmaceuticals and LFKRI of the NYBC to discover new drug therapies for the treatment of river blindness (onchocerciasis). The collaboration's goal is to identify a novel, potent macrofilaricidal drug candidate that is capable of killing adult worms.

Overlap: none

3. R01AI098775-01 MPI: Hotez/Bottazzi/Jiang; Co-PI S. Lustigman 05/01/2012 – 04/30/2017

(b)(6)

NIH/NIAID

\$273,860 (subcontract)

RBD Recombinant Protein-based SARS Vaccine for Biodefense

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Overlap: none

4. 1R01AI105431-01 MPI: Lustigman (Contact PI)/Bottazzi/Shen 1/2013 – 12/2017

(b)(6)

NIH/NIAID

\$1,203,147 (including three subcontracts)

Development of a novel adjuvant for vaccine sparring

Adjuvants are integrated into vaccines to insure their effectiveness and to support antigen sparing. Currently, alum is the only adjuvant licensed in the U.S., but it has had limited effectiveness when used with commercial flu vaccines. Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

5. OPP1086618 (Bill & Melinda Gates Foundation) (PI: S. Lustigman) 5/2013 – 10/2014

(b)(6)

\$100,000

Innovative 3-D in vitro culturing system for filarial worms

To develop 3-Dimensional *in vitro* culturing systems that supports the development of *Onchocerca volvulus* and *Brugia malayi* infective larvae to the adult stages. This will provide greater numbers of adult worms for high

throughput screening for macrofilaricidal (that kill adult worms) drugs, which are needed to support the elimination of onchocerciasis in Africa.

Overlap: none

6. OPP1099849 (Bill & Melinda Gates Foundation) (PI: S. Lustigman) 8/2013 – 4/2015
\$299,364

(b)(6)

Production of Onchocerca volvulus Larvae to Support Macrofilaricide Drug Discovery Projects

The goal is to produce at least 150,000 cryopreserved third-stage larvae of *O. volvulus* that will be used for research activities in line with the BMGF's macrofilaricidal drug discovery and development efforts.

Overlap: none

7. OPP1083910 (Bill & Melinda Gates Foundation) (PI: T. Nutman; Co-PI: S. Lustigman) 8/2013 – 8/2015
\$232,495 (subcontract with NIH Foundation)

(b)(6)

Rapid identification of individuals with viable adult female worms of Onchocerca volvulus: a means to the end

To identify host- and parasite-specific biomarker(s) present in human subjects with viable adult females of *Onchocerca volvulus* (Ov) and to develop and configure rapid point of care methods to detect (or sense) these biomarkers. This would be a final and necessary step in the progress towards elimination of onchocerciasis, an important neglected tropical disease.

Overlap: none

8. 1R56 1AI01372-01A1 MPI: Lustigman (Contact PI)/Ghedin/Unnasch 8/2013 – 7/2014
NIH/NIAID

(b)(6)

Molecular mechanisms of filarial endosymbiosis

Our goal for this project is to define the mechanisms that determine the interdependencies between the parasitic nematode *Brugia malayi* and its bacterial endosymbiont.

Overlap: none

PENDING

(b)(4)

If pending proposal is funded, appropriate adjustments will be made to ensure that Dr. Lustigman's time will total no than 100% on active projects at any given time.

E. IMPACT**E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?**

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS**F.3.a Human Subjects**

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

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SARS-CoV became a select viral agent as of December 4, 2012. UTMB has been working diligently with various regulatory agents, both on and off the UTMB campus, to comply with all regulations concerning the usage of select agents. In short, we have made an inventory of the SARS-CoV that we have in the laboratory and moved our animal (A).BSL-3 laboratories from Mary Moody Northern (MMN) into the Galveston National Laboratory complex as of December 4th, 2012 with 24/7 security service. During this transition stage our group feels strongly that there will be no negative impact on the ongoing SARS program under this grant and if any, it will be minimal. The biocontainment level for SARS-CoV remains the same, level-3, however, the antigen became classified as a select agent.

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

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G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS**G.4.a Does the project involve human subjects?**

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

G.4.c ClinicalTrials.gov**Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?**

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT**Are there personnel on this project who are newly involved in the design or conduct of human subjects research?**

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS**Does this project involve vertebrate animals?**

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: BAYLOR COLLEGE OF MEDICINE	051113330	TX-009	BAYLOR COLLEGE OF MEDICINE ONE BAYLOR PLAZA HOUSTON TX 770303411
New York Blood Center	073271827	NY-014	310 East 67 Street New York NY 100656275
The University of Texas Medical Branch	800771149	TX-014	301 University Boulevard Galveston TX 775550156
Texas Childrens Hospital	074615394	TX-009	1102 Bates Street Houston TX 770302399

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No



Environmental Health & Safety
Biological & Chemical Safety Program
Materials Management Building, 2.112
301 University Blvd.
Galveston, Texas 77555-1111
O 409.772.1781 F 409.772.8921

February 11, 2013

To Whom It May Concern

The University of Texas Medical Branch at Galveston (UTMB) is a select agent registered entity with the U.S. Health and Human Services, Centers for Disease Control and Prevention Division of Select Agents and Toxins (CDC/DSAT) and U. S. Department of Agriculture, Animal and Plant Health Inspection Service, (USDA/APHIS) National Select Agent Program. The University has been inspected by the CDC/DSAT and USDA/APHIS National Select Agent Program for use of HHS Select Agents and Toxins, Overlap Select Agents and Toxins and USDA Select Agents and Toxins.

Per the requirements of 42 CFR 73 the original certificate of registration was issued October 19, 2004, renewal was granted on April 9, 2007 and again on April 1, 2010 for use of select agents at BioSafety Levels 2, 3, and 4 and Animal BioSafety Levels 3 and 4. Inspection by CDC/DSAT and USDA/APHIS occurred January 9th to 20th 2012, for the current renewal cycle and approval was granted on March 21, 2012 for three years. The University has been registered with Health and Human Services, Centers for Disease Control and Prevention as a select agent facility since 1997. The University has a Responsible Official and four Alternate Responsible Officials.

Attached please find a copy of the University Of Texas Medical Branch certificate of registration of the possession, use and transfer of select agents and toxins. The registration number has been redacted for security purposes. The registration number will be provided at the time of an official CDC/USDA Form 2 transfer of select agents.

Please feel free to contact me should you require additional information.

Sincerely,

A handwritten signature in black ink that reads "Domenica Zimmerman". The signature is fluid and cursive.

Domenica Zimmerman
BioSafety Officer
Alternate Responsible Official
UTMB Select Agent Program

Certificate of Registration

Entity Name: **University of Texas Medical Branch**
 Address: **301 University Boulevard**
Galveston, TX 75555-0633

Registration #: **March 21, 2012**
 Effective Date: **March 21, 2015**
 Expiration Date: **March 21, 2015**

USDA

Responsible Official: **Michael Shriner**

Alternate Responsible Official(s): **Carlos Escobar, Amy Goebel, Scott Weaver, Domenica Zimmerman**

Based on information provided to the CDC Select Agent Program and the APHIS Agent Select Agent Programs, the above-named entity is authorized to possess, use, and transfer select agent and toxins under the conditions specified in the entity registration application, in accordance with 42 CFR part 73, 9 CFR part 121, and 7 CFR part 331.

Robbin S. Weyant

Robbin S. Weyant, Director
 Select Agent Program
 Centers for Disease Control and
 Prevention

Freda E. Isaac, DVM

Freda E. Isaac, DVM, Director
 Select Agent Program
 Veterinary Services

Charles L. Divan

Charles L. Divan, Branch Chief
 Select Agent Program
 Plant Protection and Quarantine



Inclusion Enrollment Report Table

This report format should NOT be used for data collection from study participants.

Study Title: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

Total Enrollment: 0

Protocol Number:

Grant Number: R01AI098775-03

PART A. TOTAL ENROLLMENT REPORT : Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
Hispanic or Latino	0	0	0	0
Not Hispanic or Latino	0	0	0	0
Unknown (Individuals not reporting ethnicity)	0	0	0	0
Ethnic Category:Total of All Subjects	0	0	0	0
Racial Categories				
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian Or Other Pacific Islander	0	0	0	0
Black Or African American	0	0	0	0
White	0	0	0	0
More than one race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of All Subjects	0	0	0	0
PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian Or Other Pacific Islander	0	0	0	0
Black Or African American	0	0	0	0
White	0	0	0	0
More than one race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics Or Latinos	0	0	0	0

Inclusion Enrollment Comments: This project is not required to enroll subjects. The research has exemption approval and only involves the collection or study of existing data, documents, records, pathological specimens or diagnostic specimens.



Grant Number: 5R01AI098775-04
FAIN: R01AI098775

Principal Investigator(s):
Maria Elena Bottazzi
PETER J HOTEZ (contact), PHD
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Leanne Brooks Scott
Business Official
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Period Of Performance:
Budget Period: 05/01/2015 – 04/30/2016
Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$1,165,726 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Vandhana Khurana
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI098775-04**Award Calculation (U.S. Dollars)**

Federal Direct Costs	\$782,695
Federal F&A Costs	\$383,031
Approved Budget	\$1,165,726
Total Amount of Federal Funds Obligated (Federal Share)	\$1,165,726
TOTAL FEDERAL AWARD AMOUNT	\$1,165,726

AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$1,165,726
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SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
4	\$1,165,726	\$1,165,726
5	\$1,165,855	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy, Immunology and Transplantation Research
CFDA Number: 93.855
EIN: 1741613878A1
Document Number: RAI098775A
PMS Account Type: G (Pooled)
Fiscal Year: 2015

IC	CAN	2015	2016
AI	8472315	\$1,165,726	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC: M51C B / **OC:** 414E / **Released:** P11 04/17/2015
Award Processed: 03/23/2015 01:36:12 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI098775-04

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 5R01AI098775-04

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Award.
- b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- c. 45 CFR Part 75.
- d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
- f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm> for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI098775. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI098775-04

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

RESTRICTION: Under governing PHS Policy, Federal funds administered by the Public Health Service (PHS) shall not be expended for research involving live vertebrate animals without prior approval by the Office of Laboratory Animal Welfare (OLAW) of an Assurance to comply with the PHS Policy on Humane Care and Use of Laboratory Animals and the project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The present award is being made without currently valid verification of IACUC approval for the portion of this project being completed in CHINA with the following restriction: No activities that involve live vertebrate animals may be conducted at Frontier Biosciences located in CHINA pending acceptance by the NIH awarding component of verification of IACUC approval. The Program Officer has approved the funding of this application without the portion of Frontier Biosciences located in CHINA in year 05 as the project is viable without it. No funds may be expended for the foreign site pending the resolution of internal administrative issues. Once these issues have been resolved, this award may be revised to include the study originally planned for the foreign site. Failure to comply with

this special condition can result in suspension and/or termination of this award, withholding of support, audit disallowances, and/or other appropriate action.

This award includes funds awarded for consortium activity with NY Blood Center.

This award includes funds awarded for consortium activity with the University of Texas Medical Branch.

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch15.htm#_Toc271265264.

No foreign performance activity may be added without prior approval of the NIAID Program and Grants Management Staff.

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at <http://www.selectagents.gov/Regulations.html>) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Jason A. Lundgren
Email: lundgrenj@mail.nih.gov **Phone:** 240-669-2973 **Fax:** 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone:** 240-627-3380

SPREADSHEET SUMMARY

GRANT NUMBER: 5R01AI098775-04

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	Year 4	Year 5
TOTAL FEDERAL DC	\$782,695	\$768,645
TOTAL FEDERAL F&A	\$383,031	\$397,210
TOTAL COST	\$1,165,726	\$1,165,855

Facilities and Administrative Costs	Year 4	Year 5
F&A Cost Rate 1	57.3%	57.3%
F&A Cost Base 1	\$668,466	\$693,211
F&A Costs 1	\$383,031	\$397,210

A. COVER PAGE

Project Title: RBD recombinant protein-based SARS vaccine for biodefense	
Grant Number: 5R01AI098775-04	Project/Grant Period: 05/04/2012 - 04/30/2017
Reporting Period: 05/01/2014 - 04/30/2015	Requested Budget Period: 05/01/2015 - 04/30/2016
Report Term Frequency: Annual	Date Submitted: 03/12/2015
Program Director/Principal Investigator Information: PETER J HOTEZ , MD PHD BA Phone number: 832-824-0502 Email: hotez@bcm.edu	Recipient Organization: BAYLOR COLLEGE OF MEDICINE BAYLOR COLLEGE OF MEDICINE 1 BAYLOR PLAZA HOUSTON, TX 770303411 DUNS: 051113330 EIN: 1741613878A1 RECIPIENT ID: 35116-N3
Change of Contact PD/PI: N/A	
Administrative Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu	Signing Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu
Human Subjects: Yes HS Exempt: Yes Exemption Number: E4 Phase III Clinical Trial:	Vertebrate Animals: Yes
hESC: No	Inventions/Patents: No

B. ACCOMPLISHMENTS**B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?**

The major goals of the project are: Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate (Timeline Year 1-3). Specific Aim 2: Process development, characterization, formulation and stability profiling (Timeline Year 2-4) and Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation (Timeline Year 4-5).

As proposed, for this reporting period activities related to Specific Aim 1 (Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate) were initiated. Specifically, we have achieved 50% completion of the activities related to the sub-specific aims 1.A. Feasibility of scalable expression, 1.B. Antigenicity and functionality and 1.C. Immunogenicity. For sub-specific aim 1.D. Efficacy, 33.3% of this activity has been completed. The goals will not change for the next reporting period and no significant changes in approach or methods are envisioned.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

Yes

Revision/ Supplements #	Revision/ Supplements Title	Specific Aims	Accomplishments
3R01AI098775-03S1	RBD recombinant protein-based SARS vaccine for biodefense	The project plan was to instruct the student on fundamental techniques in molecular biology and biochemistry and provide a broad educational overview on the key steps in developing a vaccine to prevent a major public health threat. The project plan included regular meetings with the research team; one-on-one mentorship meetings to provide feedback on weekly activities; answer questions and provide additional training if needed; and training in various general aspects of research. The student's work fell under Specific Aim 2 of the parent grant, specifically, Process development, characterization, formulation and stability profiling.	The student assisted with process development of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV), a major part of the Specific Aim 2. The student assisted with the discovery of possible purification parameters as well as the identification of in-process samples over the chromatographic purification procedure thereby contributing to Milestone 1: A suitable expression is selected for expression of rRBD in small scale and Milestone 4: Established a reproducible 10L scale process for a stable rRBD-based vaccine in preparation for future technology transfer to a cGMP manufacturing facility.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

The Contact PI was appointed as Member, Governor Rick Perry's Texas Task Force on Infectious Disease Preparedness and Response which allowed for the dissemination to communities about the project and enhance public understanding. In addition a presentation in the Civic Scientist Lecture Series, Baker Institute, Rice University (Houston, TX) was discussed the topic of "Influenza, SARS, Ebola and the

Next Pandemic: Perceptions in the Media and Public".

Co-I was asked to be a panelist for the Panel entitled "Working with International Non-Governmental Organizations (NGOs) and Not-for-Profit Organizations Against Emerging Infectious Disease and Biodefense Threats" at the 11th Annual Emerging Infectious Diseases & Biodefense Summit.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

As we move into year four, we plan to complete the optimization of the adjuvant formulation using the selected yeast-expressed, rRBD protein-based SARS vaccine candidate RBD219-N1. Formulations of the RBD219-N1 protein with glucopyranosyl lipid A (GLA)-stable-emulsion (SE) and with GLA-AF (emulsion-free) in combination with Alhydrogel® are being compared. The protein alone is being used as the control. We will assess augmentation of RBD-specific immunogenicity (IgG, IgG1, IgG2a antibody responses) and elicitation of effective neutralizing antibodies in the vaccinated mice using ELISA and pseudotyped and live SARS-CoV-based neutralization assays. The resulting adjuvanticity of the two GLA formulations on the immunogenicity of RBD219-N1 will be compared with that of Alhydrogel®, AddaVax and Advax-2 adjuvants. In addition, we plan to use any available residual funds to test for the survival of immunized mice after virus challenge and for the possibility of the development of pathology in the lungs of vaccinated mice. These experiments will enable us to select the best adjuvant/vaccine antigen formulations for cGMP manufacturing.

We are scheduling the execution of 3 successive process development runs at the 10L scale for year four. We plan to utilize the developed assays to characterize the purified protein. The technology transfer will be arranged after the three reproducible process development runs for cGMP Manufacture have been completed.

We will continue to closely coordinate with the consortium partners NYBC and UTMB. We will begin to engage in quality assurance activities with our associates at the Sabin Vaccine Institute and to coordinate efforts for technology transfer with WRAIR as we execute the year 4 activities.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

B.2 Year 3 Accomplishments.

As described in last year's report, RBD219-N1 was chosen as the SARS vaccine candidate. The work related to Aims 1A to 1D has been completed and has been reported previously. For this reporting period, the major activities performed were in support of completing Aim 1 and advancing Aim 2.

Aim 1.E. Optimization of the immunization regimen.

We have optimized the immunization regimen for the candidate SARS-CoV RBD vaccine (RBD219-N1) in a mouse model. We vaccinated mice with RBD219-N1 adsorbed to Alhydrogel[®], subcutaneously (s.c.) or intramuscularly (i.m.), three times, at 3-week intervals. Sera were collected 10 days after the last immunization and tested for IgG antibody responses and for neutralizing antibodies against SARS pseudovirus and live SARS-CoV infections.

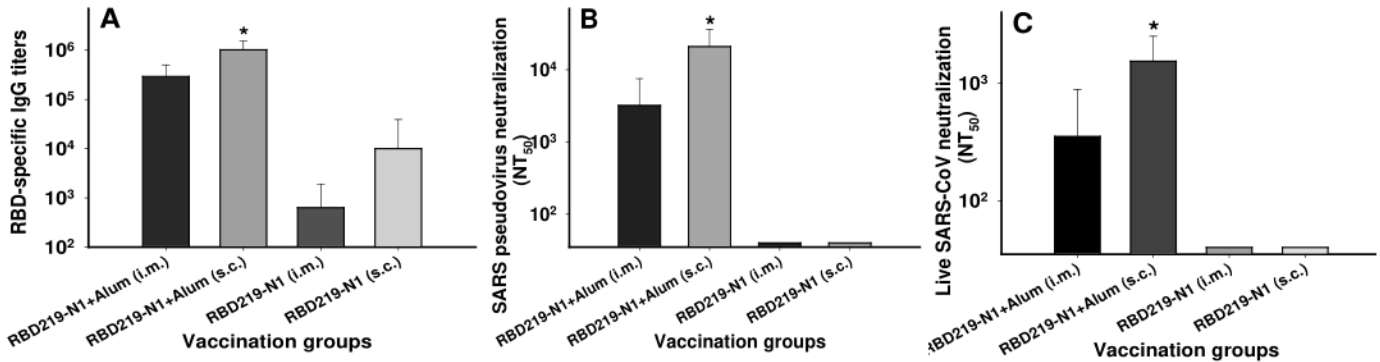


Figure 1. Optimization of immunization routes of SARS-CoV RBD protein. (A) Detection of IgG antibody response by ELISA in mouse sera. Neutralization antibody titers against SARS pseudovirus (B) and live SARS-CoV (C) in mouse sera. [Alhydrogel[®] abbreviated as Alum.]

The RBD219-N1/Alhydrogel[®] vaccine induced high titers of specific IgG (**Fig. 1A**) and neutralizing antibodies against infections of SARS pseudovirus in ACE2/293T cells (**Fig. 1B**) and live SARS-CoV in Vero cells (**Fig. 1C**) through both s.c. and i.m. routes. RBD219-N1 protein only induced low titer of IgG antibody without neutralizing activity (**Fig. 1A**). Although the antibody responses induced through s.c. route are significantly higher than that through i.m. route (**Fig. 1**), we still selected the i.m. route for subsequent adjuvant optimization because i.m. injection of the vaccines containing adjuvants has less chance to induce adverse local effects than s.c. injection (<http://vaccine-safety-training.org/route-of-administration.html>) and the majority of the clinically used vaccines are administered via i.m. route (<http://www.immunize.org/catg.d/p3085.pdf>).

To optimize additional adjuvant formulations, we first evaluated a glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), and compared its immunogenicity in mice to the RBD219-N1/Alhydrogel[®] vaccine using the same immunization scheme and analyses as described above.

Table 1. Immunogenicity of RBD219-N1 Vaccine Formulations (All vaccinations administered intramuscularly.)						
Groups	Antibody titers			Ratio	Neutralizing antibody titers (NT ₅₀)	
	IgG	IgG1	IgG2a	IgG1/IgG2a	SARS pseudovirus	Live SARS-CoV
RBD219-N1 + Alhydrogel [®]	31,962	188,054	1,566	~120	9,192	676
RBD219-N1 + GLA-SE	33,262	26,047	75,713	~0.3	<20	20
RBD219-N1 protein only	1,049	<900	<900	NA	<20	<20

As shown in **Table 1**, RBD219-N1 vaccines elicited a similarly high RBD-specific antibody responses (IgG titers > 1:30,000) when formulated with either Alhydrogel[®] or the GLA-SE adjuvant. Notably, RBD219-N1 plus Alhydrogel[®] induced a higher IgG1 but lower IgG2a antibody response (IgG1/IgG2a ratio, 1:120), whereas the IgG1 and IgG2a antibodies elicited by RBD219-N1 plus GLA-SE were at a similar level (IgG1/IgG2a ratio, 3:1). RBD219-N1 protein alone only induced significantly lower antibody responses. Importantly, RBD219-N1 plus Alhydrogel[®] also elicited neutralizing antibodies against both pseudo-typed and live SARS-CoV. In contrast, immunization with RBD219-N1 plus GLA-SE, as well as the protein alone, did not. The reason why GLA-SE could not improve the ability of RBD219-N1 to induce neutralizing antibodies is currently being investigated in a repeat study.

Aim 1.F. Ability of the rRBD-based vaccine to induce cross-neutralizing antibody responses and protection in mice.

The selected SARS-CoV RBD219-N1-Alhydrogel[®] vaccine candidate is immunogenic, inducing high neutralizing antibody titers. Importantly, mice vaccinated 3 times, 3-weeks apart, were fully protected against viral infection and mortality caused by SARS-CoV. Post-mortem examination of lungs from vaccinated and challenged mice revealed eosinophilic infiltration, which has prompted us to further explore the root cause and therefore continue evaluating alternate adjuvants for the vaccine formulation.

Specific Aim 2.A. Development and optimization of a 10 L scale process.

Aim 2.A.1. Upstream process optimization.

To improve the expression yield of RBD219-N1, several fermentation parameters were optimized at the 5 L scale, including temperature, pH, sorbitol co-feed, media salt concentration, detergents, methanol feed rate and clone copy number. For the final fermentation process, a low salt medium was inoculated with a high copy number clone, and the culture was induced at 24°C, pH 6.5 with a gradual increasing amount of the methanol flow rate from 11 to 15 mL/L/hr over 70±2 hours. This improved the yield of RBD219-N1 from, originally, 45 mg/L (**Figure 2a**) to 400 mg/L at the 10 L fermentation scale (**Figure 2b**).

Aim 2.A.2. Downstream process optimization.

We investigated three purification schemes: (1) Butyl Hydrophobic Interaction Chromatography (HIC) followed by Size Exclusion Chromatography (SEC); (2) Cation Exchange Chromatography (CEX) followed by HIC; and (3) CEX followed by SEC. Based on initial yield and purity, we discontinued schemes (2) and (3), and focused on the optimization of scheme (1). We investigated different buffer salts, binding capacity, step elution and injection volume for SEC and were able to lock down a purification scheme which met the goals of purity and yield. We were able to obtain a yield of 250 mg RBD219-N1 per L of fermentation supernatant (10 L fermentation, providing 6 L supernatant) with an overall recovery rate of approximately 45%.

Aim 2.B. Assay development.

We have developed four assays to assess the purity and identity of RBD219-N1: SDS-PAGE (reduced and non-reduced), Western Blot, HPLC-SEC and HPLC-RP. For the HPLC-RP, the column and buffer conditions were optimized. In the final assay, RBD219-N1 will be bound to a C4 column in 30% Acetonitrile/0.5% TFA and gradually eluted with Acetonitrile (2% increase/min). In addition, we also established endotoxin testing (Endosafe[®]-PTS[™], Charles River Inc.) for the purified protein. In parallel, we are currently developing a quantitative Host-cell-protein slot blot assay to evaluate residual contaminants. Some other outsourced assays, e.g., mass spectrometry, N-terminal sequencing, etc., will also be used to characterize the protein and ensure its integrity.

Aim 2.C. Execution of three successive process development runs at the 10 L scale.

As described in Aim 2.A., we continued the optimization of our upstream and downstream processes in preparation of the three successive process development runs scheduled to be performed at the start of Project year 4.

Aim 2.D. Formulation and Stabilization.

Six different formulations (**Table 2**) were prepared for the pre-clinical study. All adjuvants will be mixed with RBD at the point-of-injection (POI) with the exception of Alhydrogel[®], which is premixed. To determine the stability of the RBD formulations when GLA-AF is combined at the POI, variable amounts of GLA-AF were combined with fixed amounts of the formulated vaccines. These preparations were then analyzed at room temperature, 4 hours post mixing by Coomassie-stained reduced SDS-PAGE and BCA assay. Both the SDS-PAGE and BCA assay suggested that the RBD remains stable and that the amount of GLA-AF used would not displace RBD from the Alhydrogel[®].

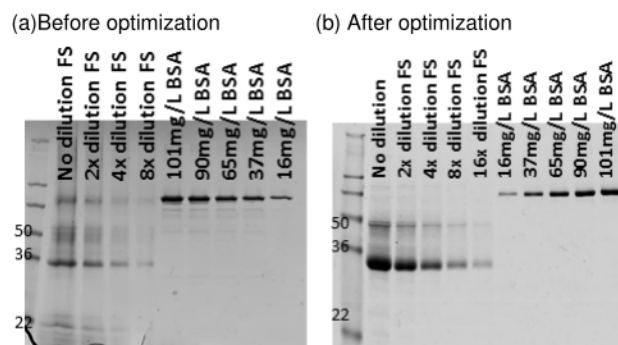


Figure 2. Quantitative gels for the fermentation supernatant (FS) before (a) and after (b) the upstream process was optimized.

Table 2. Formulations of RBD219-N1 vaccine for the pilot study. All vaccines were administered intramuscularly (100 µl/mouse).

No.	RBD219-N1 Dose	Adjuvant	Adjuvant Dose
1	1 st : 20 µg; 2 nd and 3 rd : 10 µg	Alhydrogel [®]	500 µg/mouse
2	1 st : 20 µg; 2 nd and 3 rd : 10 µg	Advax-2	1 mg/mouse
3	1 st : 20 µg; 2 nd and 3 rd : 10 µg	GLA-SE	5 µg/mouse
4	1 st : 20 µg; 2 nd and 3 rd : 10 µg	GLA-AF	5 µg/mouse
		Alhydrogel [®]	500 µg/mouse
5	1 st : 20 µg; 2 nd and 3 rd : 10 µg	AddaVax	50 µl/mouse
6	1 st : 20 µg; 2 nd and 3 rd : 10 µg	No adjuvant	--

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?**B.4. What opportunities for training and professional development has the project provided?**

Funds were received for a minority supplement on the development and manufacture of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV). The project served as a basis for engaging an under-represented minority high school student in an eight-week long mentored program of biotechnology and biochemistry research. The program was offered in association with the Office of Diversity and Community Outreach at Baylor College of Medicine.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Jiang S, Lu L, Du L, Debnath AK. Putative conformations of the receptor-binding domain in S protein of hCoV-EMC in complex with its receptor dipeptidyl peptidase-4. J Infect. 2013 Aug;67(2):156-8. PubMed PMID: 23603488; PubMed Central PMCID: PMC4355062.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING**C.5.a Other products**

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	HOTEZ, PETER J	PII		BA,PHD, MD	PD/PI	EFFORT	0	0			NA
	Y	JIANG, SHIBO			PHD,MD	PD/PI		0	0	Fudan University	CHINA	NA
	Y	Bottazzi, Maria Elena				PD/PI		0	0			NA
	N	Du, Lanying			PHD	Co-Investigator		0	0			NA
	N	Ewere, Ebe				High School Student		0	2			NA
	N	Zhang, Naru			Ph.D.	Research Fellow		0	0			NA
	N	LUSTIGMAN, SARA			PHD	Co-Investigator		0	0			NA
	N	Seid, Chris			Ph.D.	Staff scientist (Doctoral level)		0	0			NA
	N	Tricoche, Nancy			BS	Non-Student Research Assistant		0	0			NA
	N	Tseng, Chien-Te K			PHD,MS	Co-Investigator		0	0			NA
	N	Nino, Diane			BSci	Project Manager		0	0			NA
	N	Pollet, Jeroen			Ph.D.	Director, Formulation		0	0			NA
	N	Tao, Xinrong			Ph.D.	Research Associate		0	0			NA
	N	Chen, Wen			Ph.D.	Non-Student Research Assistant		0	0			NA
	N	Chan, Tehseng			MD, PhD	Co-Investigator		0	0			NA
	N	Chag, Shivali			MS	Non-Student Research Assistant		0	0			NA
	N	Hudspeth, Elissa			BS	Technician		0	0			NA

Glossary of acronyms:

S/K - Senior/Key

Foreign Org - Foreign Organization Affiliation

SS - Supplement Support

DOB - Date of Birth
 Cal - Person Months (Calendar)
 Aca - Person Months (Academic)
 Sum - Person Months (Summer)

RE - Reentry Supplement
 DI - Diversity Supplement
 OT - Other
 NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

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D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

OTHER SUPPORT AT BAYLOR COLLEGE OF MEDICINE

HOTEZ, PETER J.

Newly awarded:

Adjuvant Technologies to Advance Chagas Disease Vaccine Development

CURRENT

23386	Hotez (PI)	01/01/2011-12/31/202014	EFFORT
	Sponsor: Dutch Government	€842,857	
	Title: Product Development Support of the Human Hookworm Vaccine		
	The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the <i>Na</i> -GST-1 and <i>Na</i> -APR-1 hookworm antigens in both adults and children.		
1016395	Hotez (PI)	08/01/2011 - 04/30/2015	EFFORT
	Sponsor: Private Source		
	Title: Human Hookworm Vaccine Initiative 3	\$871,809	
	Clinical Development and Evaluation of the <i>Na</i> -GST-1 and <i>Na</i> -APR-1 Hookworm Vaccine Antigens		
	The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by <i>Necator americanus</i> .		
	Hotez (PI)	04/20/2012 – 04/19/2016	EFFORT
	Private Source	\$244,150	
	Accelerating the development and testing of a therapeutic Chagas vaccine		
	The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.		
5R01AI098775-02	Hotez/Bottazzi/Jiang (MPI)	05/04/2012 – 04/30/2017	EFFORT
	Sponsor: National Institutes of Health	\$899,886	
	Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence		
	The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.		
	Bottazzi (Center Director, Consultant)	11/01/2012 – 12/31/2017	EFFORT
	Sponsor: Department of Health and Human Services / Texas A&M Univ.	\$255,928	
	Title: Centers for Innovation in Advanced Development and Manufacturing		
	The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.		
	Role: Instructor		
	Hotez/Bottazzi (MPI)	08/01/2013 – 07/31/2017	EFFORT
	Sponsor: Private Source	\$328,435	
	Title: Multivalent Anthelmintic Vaccine Discovery Program		
	The overarching goal of this four year project is to advance the development of a lead candidate <i>Ascaris</i> antigen and a <i>Trichuris</i> antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.		
	Hotez (PI)	01/01/2014 – 12/31/2016	EFFORT
	Sponsor: University of Malaya	\$250,000	
	Title: Malaysian Neglected Tropical Disease Initiative		
	Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.		
	Hotez (PI)	01/01/2014 – 12/31/2017	EFFORT

Sponsor: Private Source \$160,000

Title: West Nile Virus vaccine development

Main goal is to support West Nile Virus vaccine development.

Hotez/Bottazzi (MPI) 01/01/2014 – 12/31/2017

EFFORT

Sponsor: Private Source \$179,348

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Bottazzi/Hotez (MPI) 07/01/2012 – 12/31/2014

EFFORT

Sponsor: Instituto Carlos Slim de la Salud \$709,333

Title: Slim Initiative for Antipoverty Vaccine Development

The main goal of this project is to build a new generation of urgently needed vaccines for the neglected diseases, and to build capacity for vaccine development in Mexico.

Hotez (PI) 11/1/2014 – 10/31/2016

EFFORT

Sponsor: Private Source

Title: Adjuvant Technologies to Advance Chagas Disease Vaccine Development \$773,754

The overall goal of this project is to develop a new vaccine formulation for Chagas disease consisting of a promising protein-based antigen (Tc24) formulated with a novel TLR4 agonist adjuvant, E6020, which is designed to skew the immune response toward a T_H1 bias and the generation of cytotoxic T cells.

BOTTAZZI, MARIA ELENA**Newly awarded:**

Adjuvant Technologies to Advance Chagas Disease Vaccine Development

ACTIVE

1016395 Hotez (PI) 08/01/2011 – 04/30/15 EFFORT
 Sponsor: Private Source
 Title: Human Hookworm Vaccine Initiative 3 \$955,573
 Clinical Development and Evaluation of the Na-GST-1 and Na-APR-1 Hookworm Vaccine Antigens
 The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.
 Role: Co-Investigator

Hotez (PI) 04/20/2012 – 12/31/2015 EFFORT
 Private Source \$244,150
 Accelerating the development and testing of a therapeutic Chagas vaccine
 The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.
 Role: Director of Product Development

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017 EFFORT
 Sponsor: National Institutes of Health \$899,886
 Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence
 The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi (Center Director, Consultant) 11/01/2012 – 12/31/2017 EFFORT
 Sponsor: Department of Health and Human Services / Texas A&M Univ. \$255,517
 Title: Centers for Innovation in Advanced Development and Manufacturing
 The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

1R01AI105431-01 Lustigman (PI) 01/15/2013 – 12/31/2017 EFFORT
 Sponsor: NIH via New York Blood Center \$177,000
 Title: Development of a novel adjuvant for vaccine sparring
 Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.
 Role: Sub-PI

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017 EFFORT
 Sponsor: Private Source \$328,435
 Title: Multivalent Anthelmintic Vaccine Discovery Program
 The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

HOOKVAC Bottazzi (PI) 10/1/2013 – 9/30/2017 EFFORT
 Sponsor: European Union via sub from (AIGHD) \$91,121
 Title: Developing and Testing a novel, low-cost, effective HOOKworm VACcine to Control Human Hookworm Infection in endemic countries
 Major goals of the project are to perform technology transfer of processes for fermentation purification and analytical testing of the human hookworm vaccine.

Hotez (PI) 01/01/2014 – 12/31/2016 EFFORT
 Sponsor: University of Malaya \$250,000
 RPPR

Title: Malaysian Neglected Tropical Disease Initiative

Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

Role: Co-I

Hotez (PI) 01/01/2014 – 12/31/2017
 Sponsor: Private Source \$160,000

EFFORT

Title: West Nile Virus vaccine development

Main goal is to support West Nile Virus vaccine development.

Role: Co-I

Hotez/Bottazzi (MPI) 01/01/2014 – 12/31/2017
 Sponsor: Private Source \$179,348

EFFORT

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Bottazzi/Hotez (MPI) 07/01/2012 – 12/31/2014
 Sponsor: Instituto Carlos Slim de la Salud \$709,333

EFFORT

Title: Slim Initiative for Antipoverty Vaccine Development

The main goal of this project is to build a new generation of urgently needed vaccines for the neglected diseases, and to build capacity for vaccine development in Mexico.

Bottazzi (Sub-PI) 11/1/2014 – 10/31/2016
 Sponsor: Private Source ¥65,398,577

EFFORT

Title: Adjuvant Technologies to Advance Chagas Disease Vaccine Development

The overall goal of this project is to develop a new vaccine formulation for Chagas disease consisting of a promising protein-based antigen (Tc24) formulated with a novel TLR4 agonist adjuvant, E6020, which is designed to skew the immune response toward a T_H1 bias and the generation of cytotoxic T cells.

OTHER SUPPORT AT NEW YORK BLOOD CENTER

Changes for Dr. Lustigman:

A pending grant from last year (b)(4) was not funded
 NIH: One pending (b)(4) was submitted
 No changes in all other eight previously funded projects

SARA LUSTIGMAN

ACTIVE

- 1. 1R01AI078314-01A2** (PI: S. Lustigman) 8/2009 – 7/2014 (+ one year no-cost extension) (b)(6)
 NIH/NIAID \$603,776 (DC)

The development of a recombinant vaccine against human onchocerciasis

A collaborative research effort focused on the preclinical research and development process that will result, through a robust screening process, with the discovery of the best 2 recombinant *O. volvulus* vaccine antigens with the highest probability for success at inducing protective immunity in humans. The vaccine will target the *O. volvulus* larvae, known to be vulnerable to host immunological attack.

Overlap: none

- 2. OPP1017584** (Bill & Melinda Gates Foundation) (PI: J. McKerrow; Co-PI: S. Lustigman) (b)(6)
 \$320,000 (subcontract) 11/1/2012 – 10/31/2015

Developing a macrofilaricidal drug for onchocerciasis using Anacor's novel oxaborole technology

A collaborative research effort between the University of California San Francisco Sandler Center, Anacor Pharmaceuticals and LFKRI of the NYBC to discover new drug therapies for the treatment of river blindness (onchocerciasis). The collaboration's goal is to identify a novel, potent macrofilaricidal drug candidate that is capable of killing adult worms.

Overlap: none

- 3. R01AI098775-01** MPI: Hotez/Bottazzi/Jiang; Co-PI S. Lustigman 05/01/2012 – 04/30/2017 (b)(6)
 NIH/NIAID \$273,860 (subcontract)

RBD Recombinant Protein-based SARS Vaccine for Biodefense

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Overlap: none

- 4. 1R01AI105431-01** MPI: Lustigman (Contact PI)/Bottazzi/Shen 1/2013 – 12/2017 (b)(6)
 NIH/NIAID \$1,077,884

Development of a novel adjuvant for vaccine sparring

Adjuvants are integrated into vaccines to insure their effectiveness and to support antigen sparing. Currently, alum is the only adjuvant licensed in the U.S., but it has had limited effectiveness when used with commercial flu vaccines. Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

- 5. OPP1086618** (Bill & Melinda Gates Foundation) (PI: S. Lustigman) 5/2013 – 10/2014 (b)(6)
 \$100,000

Innovative 3-D in vitro culturing system for filarial worms

To develop 3-Dimensional *in vitro* culturing systems that supports the development of *Onchocerca volvulus* and *Brugia malayi* infective larvae to the adult stages. This will provide greater numbers of adult worms for high throughput screening for macrofilaricidal (that kill adult worms) drugs, which are needed to support the elimination of onchocerciasis in Africa.

Overlap: none

6. 1R56 1AI01372-01A1 MPI: Lustigman (Contact PI)/Ghedin/Unnasch 8/2013 – 7/2014
NIH/NIAID \$613,492 (+ one year no-cost extension)

(b)(6)

Molecular mechanisms of filarial endosymbiosis

Our goal for this project is to define the mechanisms that determine the interdependencies between the parasitic nematode *Brugia malayi* and its bacterial endosymbiont.

Overlap: none

7. OPP1099849 (Bill & Melinda Gates Foundation) (PI: S. Lustigman) 8/2013 – 4/2015
\$299,364

(b)(6)

Production of Onchocerca volvulus Larvae to Support Macrofilaricide Drug Discovery Projects

The goal is to produce at least 150,000 cryopreserved third-stage larvae of *O. volvulus* that will be used for research activities in line with the BMGF's macrofilaricidal drug discovery and development efforts.

Overlap: none

8. OPP1083910 (Bill & Melinda Gates Foundation) (PI: T. Nutman; Co-PI: S. Lustigman) 8/2013 – 8/2015
\$267,370 (subcontract with NIH Foundation)

(b)(6)

Rapid identification of individuals with viable adult female worms of Onchocerca volvulus: a means to the end

To identify host- and parasite-specific biomarker(s) present in human subjects with viable adult females of *Onchocerca volvulus* (Ov) and to develop and configure rapid point of care methods to detect (or sense) these biomarkers. This would be a final and necessary step in the progress towards elimination of onchocerciasis, an important neglected tropical disease.

Overlap: none

PENDING

(b)(4)

If pending proposal is are funded, appropriate adjustments will be made to ensure that Dr. Lustigman's time will total no than 100% on active projects at any given time.

Changes for Drs. Shibo Jiang and Lanying Du:

Receive an R21 grant (R21 AI111152) for B. subtilis spore-delivered M2e-FP-based mucosal universal influenza vaccines (as shown above), but will keep the originally approved effort unchanged.

SHIBO JIANG**ACTIVE**

1R21AI109094-01 (Du)

12/15/2013 – 11/30/2015

EFFORT

NIH/NIAID

Critical neutralizing domain-based vaccines against new SARS-like virus hCoV-EMC

Role: Co-Investigator

Overlap: None

ACTIVE

1R21 AI111152-01 (Du)

08/05/2014 – 07/31/2016

EFFORT

NIH/NIAID

B. subtilis spore-delivered M2e-FP-based mucosal universal influenza vaccines

Role: Co-Investigator

Overlap: None

LANYING DU**ACTIVE**

1R21AI109094-01 (Du)

12/15/2013 – 11/30/2015

EFFORT

NIH/NIAID

Critical neutralizing domain-based vaccines against new SARS-like virus hCoV-EMC

Role: PI

Overlap: None

ACTIVE

1R21 AI111152-01 (Du)

08/05/2014 – 07/31/2016

EFFORT

NIH/NIAID

B. subtilis spore-delivered M2e-FP-based mucosal universal influenza vaccines

Role: PI

Overlap: None

E. IMPACT**E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?**

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS**F.3.a Human Subjects**

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

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F.3.d. Select Agents

SARS-CoV became a select viral agent as of December 4, 2012. UTMB has been working diligently with various regulatory agents, both on and off the UTMB campus, to comply with all regulations concerning the usage of select agents. In short, we have made an inventory of the SARS-CoV that we have in the laboratory and moved our animal (A).BSL-3 laboratories from Mary Moody Northern (MMN) into the Galveston National Laboratory complex as of December 4th, 2012 with 24/7 security service. During this transition stage our group feels strongly that there will be no negative impact on the ongoing SARS program under this grant and if any, it will be minimal. The biocontainment level for SARS-CoV remains the same, level-3, however, the antigen became classified as a select agent.

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

File(s) uploaded:
utmb select agent.pdf

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS**G.4.a Does the project involve human subjects?**

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

G.4.c ClinicalTrials.gov**Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?**

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT**Are there personnel on this project who are newly involved in the design or conduct of human subjects research?**

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)**Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?**

No

G.7 VERTEBRATE ANIMALS**Does this project involve vertebrate animals?**

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: BAYLOR COLLEGE OF MEDICINE	051113330	TX-009	BAYLOR COLLEGE OF MEDICINE ONE BAYLOR PLAZA HOUSTON TX 770303411
New York Blood Center	073271827	NY-014	310 East 67 Street New York NY 100656275
The University of Texas Medical Branch	800771149	TX-014	301 University Boulevard Galveston TX 775550156
Texas Childrens Hospital	074615394	TX-009	1102 Bates Street Houston TX 770302399

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE**G.10.a** Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No



Environmental Health & Safety
Biological & Chemical Safety Program
Materials Management Building, 2.112
301 University Blvd.
Galveston, Texas 77555-1111
O 409.772.1781 F 409.772.8921

February 11, 2013

To Whom It May Concern

The University of Texas Medical Branch at Galveston (UTMB) is a select agent registered entity with the U.S. Health and Human Services, Centers for Disease Control and Prevention Division of Select Agents and Toxins (CDC/DSAT) and U. S. Department of Agriculture, Animal and Plant Health Inspection Service, (USDA/APHIS) National Select Agent Program. The University has been inspected by the CDC/DSAT and USDA/APHIS National Select Agent Program for use of HHS Select Agents and Toxins, Overlap Select Agents and Toxins and USDA Select Agents and Toxins.

Per the requirements of 42 CFR 73 the original certificate of registration was issued October 19, 2004, renewal was granted on April 9, 2007 and again on April 1, 2010 for use of select agents at BioSafety Levels 2, 3, and 4 and Animal BioSafety Levels 3 and 4. Inspection by CDC/DSAT and USDA/APHIS occurred January 9th to 20th 2012, for the current renewal cycle and approval was granted on March 21, 2012 for three years. The University has been registered with Health and Human Services, Centers for Disease Control and Prevention as a select agent facility since 1997. The University has a Responsible Official and four Alternate Responsible Officials.

Attached please find a copy of the University Of Texas Medical Branch certificate of registration of the possession, use and transfer of select agents and toxins. The registration number has been redacted for security purposes. The registration number will be provided at the time of an official CDC/USDA Form 2 transfer of select agents.

Please feel free to contact me should you require additional information.

Sincerely,

A handwritten signature in black ink that reads "Domenica Zimmerman". The signature is fluid and cursive, with the first name being more prominent.

Domenica Zimmerman
BioSafety Officer
Alternate Responsible Official
UTMB Select Agent Program

Certificate of Registration

Entity Name: **University of Texas Medical Branch**
 Address: **301 University Boulevard**
Galveston, TX 75555-0633

Registration #: _____
 Effective Date: **March 21, 2012**
 Expiration Date: **March 21, 2015**

USDA

Responsible Official: **Michael Shriner**

Alternate Responsible Official(s): **Carlos Escobar, Amy Goebel, Scott Weaver, Domenica Zimmerman**

Based on information provided to the CDC Select Agent Program and the APHIS Agricultural Select Agent Programs, the above-named entity is authorized to possess, use, and transfer select agent and toxins under the conditions specified in the entity registration application, in accordance with 42 CFR part 73, 9 CFR part 121, and 7 CFR part 331.

Robbin S. Weyant

Robbin S. Weyant, Director
 Select Agent Program
 Centers for Disease Control and
 Prevention

Freda E. Isaac, DVM

Freda E. Isaac, DVM, Director
 Select Agent Program
 Veterinary Services

Charles L. Divan

Charles L. Divan, Branch Chief
 Select Agent Program
 Plant Protection and Quarantine



Inclusion Enrollment Report**Inclusion Data Record (IDR) #:** 154039**Study Title:** RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001**Foreign/Domestic:** Domestic**Planned Enrollment Report****Comments:** This project is not required to enroll subjects. The research has exemption approval and only involves the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens.

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More than One Race	0	0	0	0	0
Total	0	0	0	0	0

Cumulative Enrollment Report**NOTE:** No cumulative inclusion enrollment data exists in the previous inclusion format or modified format. Although prompted to do so, the PD/PI did not enter information in the modified format. No data can be provided.